The Role of Subchondral Bone in Osteoarthritis

by

Dawn Doré, BSc(Human Kinetics), BBiotech(Hons)

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(Medical Research)

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Supervisors **Professor Graeme Jones**

Doctor Tania Winzenberg

Doctor Stephen Quinn

Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Co-Authorship

This thesis includes papers for which Dawn Doré (DD) was not the sole author. DD was the lead in the research of each manuscript; however, she was assisted by the co-authors whose contributions are detailed below.

Chapters 4

Doré D, Quinn S, Ding C, Winzenberg T, Jones G. Correlates of subchondral BMD: A cross-sectional study. Journal of Bone and Mineral Research. 2009;24(12):2007-15.

The contribution of each author:

DD was responsible for data collection, data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions.

SQ and TW participated in analysis and interpretation of the data, and critically revised the manuscript.

CD participated in analysis and interpretation of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 5

Doré D, Quinn S, Ding D, Winzenberg T, Cicuttini F, Jones G. Subchondral bone and cartilage damage: a prospective study in older adults. Arthritis & Rheumatism. 2010;62(7):1967-73.

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DD was responsible for data collection, data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions.

SQ and TW participated in analysis and interpretation of the data, and critically revised the manuscript.

CD participated in analysis and interpretation of data, and critically revised the manuscript.

FC designed and carried out the study planning, participated in analysis and interpretation of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 6

Doré D, Martens A, Quinn S, Ding C, Winzenberg T, Zhai G, Pelletier JP, Martel-Pelletier J, Abram F, Cicuttini F, Jones G. Bone marrow lesions predict site-specific cartilage defect development and volume loss: a prospective study in older adults. Arthritis Research & Therapy. 2010;12(6):R222.

The contribution of each author:

DD was responsible for data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions.

AM participated in data management and cleaning, participated in analysis and interpretation of data, and helped with manuscript preparation.

SQ and TW participated in analysis and interpretation of the data, and critically revised the manuscript.

CD participated in analysis and interpretation of data, and critically revised the manuscript.

GZ and FA carried out data collection and critically revised the manuscript.

JPP and JMP participated in the study planning, carried out data collection, and critically revised the manuscript.

FC designed and carried out the study planning, participated in analysis and interpretation of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 7

Doré D, Quinn S, Ding C, Winzenberg T, Zhai G, Cicuttini F, Jones G. Natural history and clinical significance of MRI-detected bone marrow lesions at the knee: a prospective study in community-dwelling older adults. Arthritis Research & Therapy. 2010;12(6):R223.

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DD was responsible for data collection, data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions.

SQ and TW participated in analysis and interpretation of the data, and critically revised the manuscript.

CD participated in analysis and interpretation of data, and critically revised the manuscript.

GZ carried out data collection and critically revised the manuscript.

FC designed and carried out the study planning, participated in analysis and interpretation of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 8: Doré D, deHoog J, Giles G, Ding C, Cicuttini F, Jones G. A longitudinal study of the association between dietary factors, serum lipids and bone marrow lesions of the knee. Manuscript submitted to Arthritis Research & Therapy.

The contribution of each author:

DD and JdH are co-first authors on this paper. They were responsible for data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions. DD also collected the data for this paper.

GG contributed to the conception and design of the study and critically revised the manuscript.

CD participated in analysis and interpretation of data, and critically revised the manuscript.

FC designed and carried out the study planning, participated in analysis and interpretation of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

(Signed)_____ (Date) _____

Dawn Doré (Candidate)

(Signed)_____ (Date) _____

Graeme Jones (Primary supervisor)

Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

(Signed)_____ (Date) _____

Abstract

Osteoarthritis (OA) is a complex disease characterised by involvement of multiple tissues in the synovial joint. It is a leading cause of pain and disability in older adults. It has long been hypothesised that subchondral bone plays an important role in the development and progression of the disease. This thesis aims to investigate how subchondral bone measures of the knee such as subchondral bone mineral density (sBMD), bone size, and bone marrow lesions (BMLs) are associated with important disease outcomes in OA.

A population-based sample of older adults aged 50–80 years (51% female; mean age 62 years) participated at baseline and approximately 3.0 years later. sBMD was assessed using dual-energy x-ray absorptiometry (DXA). Cartilage volume, cartilage defects, bone area, and BMLs were determined using magnetic resonance imaging (MRI). X-ray was used to assess radiographic osteoarthritis [joint space narrowing (JSN) and osteophytes]. Blood samples were collected to assess vitamin D and high-density lipoprotein (HDL) cholesterol. Multiple questionnaires were used to assess pain, function, dietary intake, physical activity, sun exposure, and total knee replacement surgery.

The first study examined the cross-sectional correlates of sBMD and found that many factors were associated with sBMD including age, sex, body mass index (BMI), vitamin D, sun exposure, physical activity, and knee structural measures. The most novel structural measure was cartilage defects and a longitudinal study was required to address causality.

In the second study, bone area at baseline predicted cartilage defect development and cartilage volume loss. Baseline sBMD predicted cartilage defect development, which confirmed the cross-sectional findings above. These associations were independent of each other, indicating there are multiple mechanisms by which subchondral bone may lead to cartilage damage.

In the third and fourth study, 43% of participants presented with a BML at baseline with 25% improving in size and 24% worsening in size over time. Baseline BMLs predicted cartilage defect development and cartilage volume loss, suggesting BMLs may have a local effect on cartilage homeostasis. Baseline cartilage defects predicted BML progression, which may represent increased bone loading adjacent to defects. These results suggest BMLs and cartilage defects are interconnected and play key roles in knee cartilage volume loss; thus, both should be considered targets for intervention. BMLs also predicted total knee replacement surgery. A change in BML size was associated with a change in

pain, only in those participants without radiographic osteoarthritis. Importantly a decrease in BML size was associated with a decrease in pain.

In the final study, baseline energy, carbohydrate and sugar intake (but not fat) were positively associated with a change in BML size. Baseline HDL cholesterol was negatively associated with BML change.

In conclusion, this series of related studies indicate that subchondral bone plays a significant role in OA pathogenesis. Features of the subchondral bone contribute to knee pain and predict important disease outcomes such as cartilage loss and joint replacement surgery. This suggests that subchondral bone is an attractive target for therapeutic intervention in OA. Future work should consider subchondral bone treatments when developing disease-modifying OA drugs (DMOADs).

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Publications Arising from the Thesis

Chapter 4: Doré D, Quinn S, Ding C, Winzenberg T, Jones G. Correlates of subchondral BMD: A cross-sectional study. Journal of Bone and Mineral Research. 2009;24(12):2007-15.

Chapter 5: Doré D, Quinn S, Ding D, Winzenberg T, Cicuttini F, Jones G. Subchondral bone and cartilage damage: a prospective study in older adults. Arthritis & Rheumatism. 2010;62(7):1967-73.

Chapter 6: Doré D, Martens A, Quinn S, Ding C, Winzenberg T, Zhai G, Pelletier JP, Martel-Pelletier J, Abram F, Cicuttini F, Jones G. Bone marrow lesions predict site-specific cartilage defect development and volume loss: a prospective study in older adults. Arthritis Research & Therapy. 2010;12(6):R222.

Chapter 7: Doré D, Quinn S, Ding C, Winzenberg T, Zhai G, Cicuttini F, Jones G. Natural history and clinical significance of MRI-detected bone marrow lesions at the knee: a prospective study in community-dwelling older adults. Arthritis Research & Therapy. 2010;12(6):R223.

Chapter 8: Doré D, deHoog J, Giles G, Ding C, Cicuttini F, Jones G. A longitudinal study of the association between dietary factors, serum lipids and bone marrow lesions of the knee. Manuscript submitted to Arthritis Research & Therapy.

Scientific Presentations Arising from the Thesis

International

2009 Osteoarthritis Research Society International (OARSI) World Congress

Montreal, Canada

"Natural history of areal bone marrow lesions" (Moderated poster presentation)

2009 3rd International Osteoarthritis Imaging Workshop

York, England

"Which subchondral bone measure is the best predictor of cartilage damage?"

(Oral presentation)

2009 2nd Joint Meeting of the International Bone & Mineral Society (IBMS) and the Australian & New Zealand Bone & Mineral Society (ANZBMS)

Sydney, Australia

"Which subchondral bone measure is the best predictor of cartilage damage?"

(Poster presentation)

National

2008 Australian & New Zealand Bone & Mineral Society (ANZBMS) Conference

Melbourne, Australia

"Determinants of subchondral bone mineral density: a cross-sectional study"

(Poster presentation)

2010 Australian Rheumatology Association (ARA) Conference

Melbourne, Australia

"Natural history and clinical significance of bone marrow lesions in osteoarthritis"

(Poster presentation)

Local

2008 Sharing Excellence in Research (SEiR), University of Tasmania, Conference
Hobart, Australia
"Determinants of subchondral bone mineral density: a cross-sectional study"
(Oral presentation)

2010 Sharing Excellence in Research (SEiR), University of Tasmania, Conference
Hobart, Australia
"Bone marrow lesions; what are they and how do they relate to osteoarthritis?"
(Oral presentation)

Invited Speaker

2010 Australian Physiotherapy Association (APA) State Conference
Launceston, Australia
"Natural history and clinical significance of bone marrow lesions in osteoarthritis"

2010 Exercise and Sports Science Australia Seminar
Hobart, Australia
"Bone marrow lesions in knee osteoarthritis"

Awards Resulting from the Thesis

2008 Endeavour International Postgraduate Research Scholarship (EIPRS)

2008 Tasmanian Postgraduate Research Scholarship (TPRS)

2008 Most outstanding presentation in the 'Population and Health' theme area at the Sharing Excellence in Research (SEiR), University of Tasmania, Conference

2008 Travel grant to attend the Australian & New Zealand Bone & Mineral Society (ANZBMS) Conference

2009 Travel grant to attend the 2nd Joint Meeting of the International Bone & Mineral Society (IBMS) and the Australian & New Zealand Bone & Mineral Society (ANZBMS)

2010 Winner of the Australian Society for Medical Research (ASMR) Postgraduate Student Competition

2010 Osteoarthritis Research Society International (OARSI) Exchange Scholarship

List of Abbreviations

25(OH)D	25-hydroxyvitamin D
2D	two-dimensional
3D	three-dimensional
ACR	American College of Rheumatology
ASU	avocado-soybean unsaponifiables
AQoL	assessment of quality of life
BMD	bone mineral density
BME	bone marrow edema
BMI	body mass index
BML	bone marrow lesion
BMP-7	bone morphogenetic protein 7
CI	confidence interval
CTX-II	type II collagen C-terminal telopeptide
CV	coefficient of variation
dGEMRIC	delayed gadolinium-enhanced magnetic resonance imaging of cartilage
DMOAD	disease-modifying osteoarthritis drug
DXA	dual-energy x-ray absorptiometry
FDA	Food and Drug Administration
FFQ	food frequency questionnaire
FS	fat suppressed
FSE	fast spin echo
GAG	glycosaminoglycan
GEE	generalised estimating equations
GRE	gradient-recalled echo
HDL	high-density lipoprotein
ICC	intraclass correlation coefficient

IL	interleukin
JSN	joint space narrowing
JSW	joint space width
KJ	kilojoule
K/L	Kellgren and Lawrence
KOOS	Knee injury and Osteoarthritis Outcome Score
LDL	low-density lipoprotein
LF	lateral femoral
LSC	least significant criterion
LT	lateral tibial
MF	medial femoral
MMP	matrix metalloproteinase
MOST	Multicentre Osteoarthritis Study
MRI	magnetic resonance imaging
MT	medial tibial
NHMRC	The National Health and Medical Research Council
NSAIDS	non-steroidal anti-inflammatory drugs
OA	osteoarthritis
OR	odds ratio
pa	per annum
RA	rheumatoid arthritis
RCT	randomised controlled trial
ROA	radiographic osteoarthritis
ROI	region of interest
sBMD	subchondral bone mineral density
SD	standard deviation
STIR	short tau inversion recovery

TASOAC	Tasmanian Older Adult Cohort study
TKR	total knee replacement
US	United States
VAS	visual analog score
WOMAC	Western Ontario and McMasters Universities Osteoarthritis Index
WORMS	Whole-Organ magnetic resonance imaging score
ZA	zoledronic acid

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Synopsis

Osteoarthritis (OA) is the most common form of arthritis and a leading cause of musculoskeletal pain and disability. By 2020, it is estimated that one in ten Australians will have OA. Although its signature pathologic feature is articular cartilage loss, OA involves many other joint structures including subchondral bone, ligaments, menisci, periarticular muscles, peripheral nerves, and synovium. It was hypothesised many years ago that subchondral bone plays an important role in OA pathogenesis. There is emerging evidence to support this; however, it remains controversial whether bone changes precede cartilage damage. This thesis examines the role that subchondral bone plays in OA using data from a prospective population-based study of community-dwelling older adults. Specifically, it investigates how different subchondral bone measures such as subchondral bone mineral density (sBMD), bone size, and bone marrow lesions (BMLs) relate to disease progression and disease severity. This Synopsis presents an overview of the content of each chapter.

Chapter 1 provides an overview of OA with a focus on the knee. A working definition is provided and the economic impact, burden of disease, symptoms, risk factors, and treatment and management options are discussed. A detailed description of the radiographic and clinical criteria for the diagnosis of knee OA is presented. Lastly, this chapter presents an overview of how subchondral bone relates to OA and provides a rationale for examining sBMD, bone size, and BMLs.

Chapter 2 lists the research questions to be addressed in the thesis.

Chapter 3 describes the Tasmanian Older Adult Cohort (TASOAC) study population and its design, as well as the protocols for measurement of factors which are common to multiple chapters in this thesis. Additional factors which are unique to each chapter are described in more detail within the methodology section of the subsequent chapters.

Chapter 4 describes the cross-sectional relationship between tibial sBMD and anthropometric, lifestyle, and structural measures in 740 TASOAC participants (mean age 62 years, range 50–80 years, 52% female). Medial tibial sBMD was assessed by dual-energy x-ray absorptiometry (DXA) at two regions of interest (ROI). Study factors examined included anthropometrics, vitamin D, steps/day by pedometer; joint space narrowing (JSN) and osteophytes (by x-ray); cartilage defects, cartilage volume, and

BMLs (by magnetic resonance imaging (MRI)); and hip and spine BMD (by DXA). sBMD using ROI 1 was negatively associated with age and female sex and positively associated with body mass index (BMI). In multivariable analysis, sBMD was positively correlated with steps/day (r = 0.08, *P* = 0.025), tibial osteophytes (r = 0.08, *P* = 0.028), JSN (r = 0.11, *P* < 0.01), cartilage defects (r = 0.16, *P* < 0.01), cartilage volume (r = 0.12, *P* = 0.01), BMLs [r = 0.17, *P* = 0.013 (tibial); r = 0.16, *P* = 0.018 (femoral)] and hip and spine BMD (r = 0.36, *P* < 0.01; r = 0.38, *P* < 0.01, respectively). Similar associations were observed using ROI 2, with vitamin D also associated with sBMD (r = 0.10, *P* < 0.01). In conclusion, this study identified a large number of factors associated with sBMD, of which the most novel is cartilage defects. Longitudinal studies are required to address causality.

Chapter 5 describes the association between baseline tibial bone area and tibial sBMD with tibial cartilage defect development and cartilage volume loss. A total of 341 TASOAC subjects (mean age 63 years, range 52–79 years, 48% female) provided complete data for baseline bone area and sBMD and changes in tibial cartilage volume and cartilage defects over approximately 2.7 years. In multivariable analysis, baseline bone area positively predicted cartilage defect development at the medial and lateral tibial sites (odds ratio (OR) 1.6 per one standard deviation (SD) increase, *P* < 0.01; OR 2.4 per one SD increase, *P* < 0.01, respectively) and cartilage volume loss at the medial tibial site (β = -34.9 per one SD increase, *P* < 0.01). In contrast, baseline sBMD positively predicted cartilage defect development at the medial tibial site only (OR 1.6 per one SD increase, *P* = 0.04) and was not associated with cartilage loss. In conclusion, bone area predicted medial and lateral cartilage defect development and medial cartilage volume loss, while sBMD predicted medial defect development but not cartilage loss. These associations were independent of each other indicating there are multiple mechanisms by which subchondral bone may lead to cartilage damage.

Chapter 6 determined: 1) whether baseline BML presence and/or severity predicts site-specific cartilage defect progression and cartilage volume loss; and 2) whether baseline cartilage defects predict site-specific BML progression. A total of 405 TASOAC subjects (mean age 63 years, range 52–79 years, 48% female) provided complete data for changes in cartilage volume, cartilage defects, and BMLs over approximately 2.7 years. Cartilage volume, cartilage defects, and BMLs were measured at the medial tibial (MT), medial femoral (MF), lateral tibial (LT), and lateral femoral (LF) sites. At all four sites, baseline BML presence predicted cartilage defect progression (OR 2.4–6.4, all *P* < 0.05),

and cartilage volume loss (-0.9 to -2.9% difference per annum, all $P < 0.05$) at the same site. In multivariable analysis, there was a significant relationship between BML severity and cartilage defect progression at all four sites (OR 1.8–3.2, all $P < 0.05$) and BML severity and cartilage volume loss at the MF, LT, and LF sites (β = -22.1 to -42.0, all $P < 0.05$). Additionally, baseline cartilage defect severity predicted BML progression at the MT and LF sites (OR 3.3–3.7, all $P < 0.01$). Lastly, there was a greater increase in cartilage volume loss at the MT and LT sites when both larger defects and BMLs were present at baseline (all $P < 0.05$*)*. In conclusion, baseline BMLs predicted site-specific cartilage defect progression and cartilage volume loss in a dose-response manner suggesting BMLs may have a local effect on cartilage homeostasis. Baseline cartilage defects predicted site-specific BML progression, which may represent increased bone loading adjacent to defects. These results suggest BMLs and cartilage defects are interconnected and play key roles in knee cartilage volume loss; thus, both should be considered targets for intervention.

Chapter 7 describes the natural history of BMLs at the knee using a quantitative measure and examines the association of BMLs with pain, function and stiffness scores, and total knee replacement (TKR) surgery. A total of 395 TASOAC subjects (mean age 63 years, range 52–79 years, 51% female) provided complete data for changes in BMLs, pain, function, and stiffness scores over approximately 2.7 years and total knee replacement (TKR) surgery data at approximately 5 years. BMLs were determined by measuring the maximum area of the lesion. Reproducibility for this method of measurement was excellent (intraclass correlation coefficient (ICC) 0.97). Pain, function, and stiffness were assessed by Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index scores. At baseline, 43% (n=168/395) had a BML. Of these 25% decreased in size and 24% increased. Of the remaining sample (n=227), 7% developed a new BML. In a multivariable model, a change in BML size was associated with a change in pain and function scores (β = 1.13–2.55 per 1 SD increase, all $P < 0.05$), only in those participants without radiographic osteoarthritis (ROA). Lastly, baseline BML severity predicted TKR surgery (OR 2.10/unit, $P = 0.019$). In conclusion, BMLs (assessed by measuring maximal area) were not static, with similar proportions both worsening and improving. A change in BML size was associated with changes in pain in those without established ROA. This finding suggests that fluctuating knee pain may be attributable to BMLs in those participants with early stage disease. Baseline BMLs also predicted TKR surgery. These

findings suggest therapeutic interventions aimed at altering the natural history of BMLs should be considered.

Chapter 8 describes the association between dietary factors, serum lipids and BMLs. A total of 394 TASOAC subjects (mean age 63 years, range 52–79 years, 50% female) provided complete baseline dietary data, serum lipid measurements and changes in BMLs over approximately 2.7 years later. Nutrient intake (total energy, fat, carbohydrate, protein and sugar) was assessed by food frequency questionnaire (FFQ) and blood samples were collected to assess serum lipids. Cross-sectionally, dietary factors and lipids were not significantly associated with BMLs. Baseline energy, carbohydrate and sugar intake (but not fat) were positively associated with a change in BML size (β = 13.57–19.13 mm^2 per 1 SD increase, all $P < 0.05$). High-density lipoprotein (HDL) cholesterol at baseline was negatively associated with BML change (β = -13.48 mm^2 per 1 SD increase, $P = 0.045$). In conclusion, energy, carbohydrate and sugar intake may be risk factors for BML development and progression. HDL cholesterol seems protective against BMLs. These results suggest macronutrients and lipids may be important in BML etiology and that dietary modification may alter BML natural history.

Chapter 9 summarises the findings of the thesis and also provides a number of potential directions for future research based on these conclusions.

Chapter 1 - Introduction

1.1 Epidemiology of Osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis and is one of the leading causes of pain and disability among older adults. It is a chronic localised joint disease and commonly involves weight-bearing joints such as the knees, hips, or spine, with hands and neck also being frequently affected sites. In the version 2 estimates for the Global Burden of Disease 2000 study, published in the World Health Report 2002 (1), OA is the fourth-leading cause of years lost due to disease at the global level.

In 2007, Access Economics estimated that there were 1.62 million Australians with OA (2). Although OA is an incredibly prevalent condition, it is becoming even more prevalent with the combined effects of an ageing and increasingly obese society. By 2050, it is projected there will be 3.1 million Australians or 11% of the population with OA (2).

1.1.1 History

OA was first found in our earliest known ancestors, the Java man (ca 500,000 years ago) (3). It was not clinically separated from other arthritides such as rheumatoid arthritis (3) until 1859. In 1888 OA received its name from John Kent Spender, a physician in Bath. The first radiological description that separated OA from rheumatoid arthritis came from ‘skiagrams’ in 1904 by Goldwaite (4). The definition of OA has evolved over the course of the 20th century and it is increasingly recognised that OA is a disease of the whole joint. Although its signature pathologic feature is articular cartilage loss, it commonly involves many other joint structures including subchondral bone, ligaments, menisci, periarticular muscles, peripheral nerves, and synovium (5). The failure of these joint tissues results in symptoms of pain, stiffness and functional disability (6).

1.1.2 Economic impact and disease burden

Arthritis and other musculoskeletal conditions were the fourth largest contributor to direct health expenditure in 2004-05 in Australia, amounting to $4 billion or more than 7% of total allocated health expenditure (7). OA accounted for nearly one-third ($1.2 billion) of expenditure for these conditions, and 75% of the funds allocated to OA were spent on hospital costs, mainly related to knee and hip replacements. Over 37,000 total knee and hip replacement procedures were performed in 2004-05, each costing an average of $14,000–$29,500. In 2006-07 this increased to over 40,000 procedures, each costing between $15,000–$31,900.

While the mortality rate for OA is low, there is also a cost in terms of burden of disease. The pain and disability patients experience can lead to a loss of health and wellbeing, loss of leisure time, and a decreased quality of life. This further contributes to the costs of OA through the loss of production to the economy, increased absenteeism, reduced work capacity and performance, and reduced labour force participation as a result of the related disease morbidity. Arthritis is more common in older people; therefore, a progressively ageing population is likely to further add to the associated disease burden and the cost of providing health services in Australia.

1.1.3 Symptoms

Pain is the most common symptom in OA and the usual reason for seeking medical advice (8). Patients also experience joint stiffness, tenderness, inflammation, crepitus, instability, and muscle weakness (9, 10). These symptoms lead to a limitation of movement, physical and psychological disability, and impaired quality of life (10). Patients can experience major difficulty with daily activities including walking, stair-climbing, and housekeeping (10).

Late stage OA is often characterised by both structural damage and symptomatic reports of joint pain, stiffness, and disability (11). However there is only a weak correlation between symptoms, such as pain and functional impairments and radiological change, especially in the earlier stages of disease (12, 13). There is also data to suggest that knee pain itself can modify the appearance of joint space width (JSW) in radiographs (14). Unfortunately this has plagued disease modification therapy in OA and treatments for OA largely target symptoms.

1.1.4 Risk factors

For many years OA was thought to be a degenerative joint disease resulting from ‘wear and tear’ and the inevitable effects of aging. It is now widely accepted that OA has a multifactorial aetiology and is the result of many systemic and local factors. Risk factors vary for different joints, for different stages of disease, and for the development versus the progression of the disease (15).

Age remains one of the strongest risk factors for all joints (16-18). Women are more likely to have knee and hand OA than men and also have more severe OA (19). Several studies have shown that OA is inherited and may vary by joint site. The heritable

component of OA has been estimated in twin and family studies to be between 50% and 65% with larger genetic influences for hand and hip OA than knee OA (20-22).

The prevalence and patterns of joints affected by OA vary among racial and ethnic groups (15). Chinese participants in the Beijing Osteoarthritis Study reported less hip and hand OA compared to Caucasians in the Framingham Study (23, 24). However, Chinese women had significantly higher prevalence of knee OA (25). The Johnson County Osteoarthritis Project have shown that the prevalence of hip OA in African American women was similar to that in white women, but the prevalence was slightly higher in African American men compared to white men (26).

There is increasing interest to explore the role of diet in OA. Several studies have linked low vitamin D to both hip and knee OA (27-29). In the Framingham Study subjects with a low vitamin D (<34 ng/mL) had a threefold increased risk for knee OA progression compared to those with higher vitamin D levels (≥34 ng/mL) (27). In the Study of Osteoporotic Fractures, women with a low vitamin D (<30 ng/mL) were 3 times as likely to develop incident hip OA compared to those with higher vitamin D levels (≥30 ng/mL) (29). Randomised, placebo-controlled trials are underway to evaluate vitamin D supplementation in OA. Vitamin C and vitamin K have also been linked to OA (30, 31). Low vitamin C dietary intake is associated with progressive knee OA (30) and high levels of vitamin K is associated with low prevalence of hand OA (31).

Being overweight or obese are strong risk factors for the development of OA, especially for knee OA (16). Obesity has been shown to be more important than other potential risk factors such as heredity, for knee OA (18, 32). The relationship between being overweight and hip OA is inconsistent and weaker than has been shown for knee OA (33, 34). However there is evidence to suggest that a high BMI is strongly associated with an increased risk of total hip replacement therapy (35).

Previous joint injury is a strong risk factor for OA. Transarticular fracture, meniscal tear requiring meniscectomy or anterior cruciate ligament injury can result in increased risk of knee OA development and musculoskeletal symptoms (36, 37). Additional risk factors include occupation (e.g. one involving repetitive knee bending), and mechanical factors such as adduction moment, malalignment, and muscle weakness.

As demonstrated there are multiple identified risk factors for OA. The racial and ethnic differences may be related to anatomical or genetic differences. The increase in OA in women suggests that hormonal factors may play a role in the development of OA. Increased load on the joint is most likely the primary mechanism by which being overweight or obese can cause knee and hip OA; however, systemic factors may also be

involved. Risk factor heterogeneity makes OA a difficult disease to treat and new therapies should consider targeted treatment groups in order to maximise treatment benefits.

1.1.5 Treatment and management

Numerous evidence based guidelines have been developed for the treatment of OA by a number of scientific societies and health care organisations (38-46). Table 1.1 displays a summary of these guidelines. Despite some differences in quality, there is a general consistency amongst the recommendations (47) and they mostly cater for specific users, such as clinicians, countries, or health care organizations. Treatments for OA include non-pharmacological, pharmacological, or surgical.

Table 1.1. Summary of evidence based guidelines for the treatment of OA

Year	Guideline
2000 (42)	American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update
2003 (38)	EULAR Recommendations: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT)
2003 (46)	Clinical practice guidelines for physical therapy in patients with osteoarthritis of the hip or knee. Royal Dutch Society for Physical Therapy
2005 (39)	EULAR evidence based recommendations for the management of hip osteoarthritis: report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT)
2007 (40)	EULAR evidence based recommendations for the management of hand osteoarthritis: report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT)
2009 (41)	OARSI recommendations for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009
2009 (44)	Treatment of osteoarthritis of the knee (nonarthroplasty)
2009 (45)	The Royal Australian College of General Practitioners. Guidelines for the non-surgical management of hip and knee osteoarthritis
2010 (43)	American Academy of Orthopaedic Surgeons (AAOS) clinical practice guideline on the treatment of osteoarthritis of the knee

EULAR: European League Against Rheumatism
OARSI: Osteoarthritis Research Society International

Table 1.2 provides an overview of the current Australian guidelines for the non-pharmacological and pharmacological treatments for knee and hip OA, endorsed by The Royal Australian College of General Practitioners (45). These guidelines are approved and supported by NHMRC (The National Health and Medical Research Council). Each treatment recommended has been rated based on the NHMRC's body of evidence assessment matrix (48). Grades are given based on a summation of components which include volume of evidence, consistency, clinical impact, generalisability, and applicability.

Table 1.2. Summary of the current Australian guidelines for the non-pharmacological and pharmacological treatments for knee and hip OA, endorsed by The Royal Australian College of General Practitioners (45)

Treatment	Grade of Evidence
Pharmacological therapy	
Simple Analgesia (paracetamol)	Grade A
Oral NSAID/COX-2	Grade B
Intra-articular corticosteroid	Grade B
Topical NSAIDS	Grade C
Weak and strong opioids	Grade A
Viscosupplemtation (5–13 weeks for knee OA)	
Non-pharmacological therapy	
Weight reduction	Grade B
Land based exercise	Grade B
Aquatic exercise	Grade C
Multimodal physical therapy	Grade C
Tai Chi	Grade C
Self management education programs	Grade C
Thermotherapy	Grade C
TENS	Grade C
Acupuncture	Grade C
Patellar taping (knee OA)	Grade D
Massage therapy	Grade D
Low level laser therapy	Grade D

OA: Osteoarthritis; NSAID: non-steroidal anti-inflammatory drugs; COX-2: cyclooxygenase-2 selective inhibitor; TENS: transcutaneous electrical nerve stimulation.
Body of evidence assessment matrix:
Grade A (Excellent evidence, body of evidence can be trusted to guide practice)
Grade B (Good evidence, body of evidence can be trusted to guide practice in most situations)
Grade C (Satisfactory, some evidence, body of evidence provides some support for recommendation(s) but care should be taken in its application)
Grade D (Poor, weak evidence, body of evidence is weak and recommendation must be applied with caution)

In summary, current pharmacological treatments for OA are mostly palliative and are concerned with controlling pain and improving function and quality of life. Analgesic and non-steroidal anti-inflammatory drugs (NSAIDs) are often prescribed in standard medical practice to reduce pain and control joint inflammation. These often prove to be only moderately effective, with >75% of patients reporting need for additional symptomatic treatment (49). There is an emphasis on non-pharmacological treatments for OA including lifestyle modification, physiotherapy, and exercise (10). Weight reduction and strategies to avoid weight gain are recommended for knee and hip OA (50). There is strong evidence to show that exercise has beneficial effects on pain and function, and a combination of strengthening, aerobic and functional exercise is recommended (10, 51). Exercise helps to strengthen muscles, increase range of motion, maintain joint position, control weight, and improve proprioception, balance, and cardiovascular fitness (52-55). Importantly, exercise has similar effects to analgesic and anti-inflammatory medications, but has fewer contraindications and adverse effects than drugs and surgery (41). Although exercise has proven to be a very effective treatment for OA, it has not been shown to modify disease progression (10). Additional physiotherapy treatments which have shown to be effective in the treatment of OA include taping, bracing, wedge insoles, and manual therapy (51). Surgery is recommended for debilitating pain and major functional impairments with factors such as walking, working, sleeping (56-58). Total knee and hip replacement are common for advanced disease when conservative treatments are ineffective (45, 59).

Despite OA's large disease burden there are no treatments which stop or delay the progression of the disease. Current FDA-approved treatments directed at reducing OA symptoms have not been shown to prevent ongoing joint structural damage (5). For this reason it has been suggested that treatment of the structural changes at a joint level should be assessed separately to patient-reported symptoms. In a review by Lane et al (5) it is stated that future pharmacotherapy for OA should be considered to be 'structure modifying', 'symptom modifying', or both.

1.2 Knee osteoarthritis

The knee is the weight bearing joint most commonly affected by OA (10). Studies in the UK report that the knee is the site most affected by pain in older adults, where it is typically attributed to OA in this age group (60, 61). In the Framingham Study cohort, knee OA was a leading cause of chronic disability in older persons (62). The research conducted

in this thesis focuses on knee OA and unless otherwise stated the remainder of the literature review will discuss OA at this site.

Knee OA is commonly defined by structural pathology, such as on a radiograph, and clinically using joint symptoms (63). Many studies combine both of these for the purpose of epidemiologic investigation, using the term symptomatic radiographic osteoarthritis (ROA) (64-66).

1.2.1 Radiographic criteria

Since 1904, radiographic descriptions of OA have evolved and were first defined in an atlas by Kellgren and Lawrence (K/L) in 1953 (67, 68). Their composite grading system is commonly used today and is displayed in Table 1.3. In epidemiologic and clinical studies the cut-off point of K/L two or more comprises the radiological definition of OA (68). Figure 1.1 highlights tibiofemoral joint space narrowing (JSN) and osteophytes on a radiograph.

Although new techniques have become available, conventional radiographs remain the gold standard for the diagnosis and evaluation of knee OA. Numerous atlases have been developed to be used as guides to evaluate individual radiographic features in OA at many different anatomical sites (69-77). They have great value in screening patients to confirm OA and staging OA. They include both semi-quantitative examination of individual radiographic features, or direct measurement of the interbone distance as an indicator of joint space (i.e. joint space width (JSW)) (15). Key features measured include osteophytes, JSN, subchondral sclerosis, and bony attrition. Table 1.4 summarises these atlases.

The research conducted in this thesis used the atlas from the Osteoarthritis Research Society International (OARSI) which was first published in 1996, by Altman et al (77). A revised version was published in 2007 (73) and is summarised in Table 1.5. The Altman atlas has many advantages. It provides clinicians and researchers with a standardised semi-quantitative methodology for radiographic features. It has relevance to both cross-sectional and longitudinal studies. It also has widespread accessibility. Images are available in electronic format which facilitate multi-centre studies and allows for comparison at multiple sites and for use with newer radiographs acquired in a digital format. The K/L scoring systems remains the only scoring system which uses a global score. However, it has been critised based on its emphasis on osteophytes, the mixing of distinct constructs (osteophytes, JSN, subchondral sclerosis, subchondral bone shape

changes, cysts, etc). The scale is also non-linear and therefore insensitive to changes over time (73, 78).

Table 1.3. Kellgren and Lawrence (K/L) grading system

Definition grades	Description
Grade 0: No osteoarthritis	No osteoarthritis
Grade 1: Doubtful	Doubtful narrowing of joint space and possible osteophytic lipping
Grade 2: Mild	Definite osteophytes and possible narrowing of joint space
Grade 3: Moderate	Multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
Grade 4: Severe	Large osteophyte, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends

Figure 1.1. Radiographic evidence of knee OA, including medial JSN and osteophytes.

Table 1.4. Atlases developed for the radiographic assessment of osteoarthritis

Author and year	Atlas Title
Spector et al, 1992 (69)	A Radiographic Atlas of Knee Osteoarthritis
Altman et al, 1987 (70)	Radiographic assessment of progression in osteoarthritis
Kallman et al, 1989 (71)	New radiographic grading scales of osteoarthritis of the hand
Scott et al, 1993 (72)	Reliability of grading scales for individual radiographic features of osteoarthritis of the knee: the Baltimore longitudinal study of aging atlas of knee osteoarthritis
Altman et al, 1995 (77)	Atlas of individual radiographic features in osteoarthritis
Altman et al, 2007 (73)	Atlas of individual radiographic features in osteoarthritis, revised
Verbruggen et al, 1996 (74)	Numerical scoring systems for the anatomic evolution of osteoarthritis of the finger joints
Nagaosa et al, 2000 (75)	Development of a logically devised line drawing atlas for grading of knee osteoarthritis
Croft et al, 2005 (76)	An introduction to the atlas of standard radiographs of arthritis

Table 1.5. Individual radiographic features measured by the OARSI atlas (77) for tibiofemoral osteoarthritis

Feature	Score
Marginal osteophytes	
Medial femoral condyle	0–3
Medial tibial plateau	0–3
Lateral femoral condyle	0–3
Lateral tibial plateau	0–3
Joint space narrowing	
Medial compartment	0–3
Lateral compartment	0–3
Other	
Medial tibial attrition	Absent/present
Medial tibial sclerosis	Absent/present
Lateral femoral sclerosis	Absent/present

OARSI: Osteoarthritis Research Society International

1.2.2 Clinical criteria

There are many definitions for symptomatic knee OA. One of the most common is regarded as frequent knee symptoms defined as "pain, aching, or stiffness in or around the knee on most days" for at least one month during the past 12 months (79). This definition has been used in many epidemiologic studies (80, 81). Other definitions of symptomatic knee OA may include a particular level of pain reported on a Visual Analog Score (VAS), the Knee injury and Osteoarthritis Outcome Score (KOOS) (82) or the Western Ontario and McMasters Universities Osteoarthritis Index (WOMAC) (83).

1.2.3 X-ray versus MRI

Radiographs continue to be the reference standard in many epidemiologic and clinical studies to define OA and monitor disease progression. This is due to their feasibility and tradition and the fact that scoring and measurement systems have been refined and standardised (84). However, the continued use of radiographs as an outcome measure in OA research has been criticised.

There is widespread belief that a high discordance exists between clinical and radiographic knee OA (12). Many studies have shown that radiographic features such as JSN and osteophytes do not correlate well with clinical symptoms (6, 13, 85-87). Hannan et al (85) found that less than 50% of people with evidence of OA on plain radiographs have symptoms related to these findings. In a systematic review, Bedson et al (12) reported that the proportion of knee pain found to have ROA ranged from 15–76% and in those with ROA the proportion with pain ranged from 15–81%. They reported that considerable variation occurred depending on the x-ray view, pain definition, OA grading scale, and demographic factors.

Radiographs are also insensitive to early disease, given that by the time ROA is detected, 10% of knee cartilage has already been lost (88). Early is a relative term and yet to be defined in OA. However, 'early' in this context means changes which occur prior to the development of gross abnormalities (for example, cartilage loss). Early also refers to abnormalities which occur when OA is first becoming established.

JSN is one of the main features measured on radiographs. It can be influenced by many joint structures including cartilage and meniscus, which cannot be separated by radiographs. JSN is also insensitive to change over time and requires large studies of 18-24 months' duration to detect significant changes (6). Therefore the continued use of plain radiography to define disease and monitor progression has many limitations.

Magnetic resonance imaging (MRI) has proven to be an important tool in OA research and has revolutionised the understanding of OA pathology. It allows precise visualisation of joint structures including bone, cartilage, menisci, synovium, and ligaments. While costly, it is free of ionising radiation and has good tissue contrast and anatomical resolution (89) allowing for non-invasive examination of joint structures. MRI is a useful tool to study pre-disease or early stages of disease and there is increasing evidence to demonstrate that structural change can be measured both reliably and with good responsiveness on MRI (90). Although it has not been formally accepted by regulatory authorities, there is great interest to use MRI for assessing diagnostic status, disease severity and monitoring progression in OA (78, 91).

MRIs of OA features can be measured semi-quantitatively or quantitatively with either morphological or compositional measurements (78). Semi-quantitative scoring of MRIs allows for multifeature assessment of the knee. Features which are currently believed to be relevant include articular cartilage integrity, subchondral bone marrow abnormalities, subchondral cysts, subchondral bone attrition, osteophytes, meniscal integrity, cruciate and collateral ligament integrity, synovitis/effusion, intraarticular loose bodies, and periarticular cysts/bursitis (78). Broadly speaking, semi-quantitative assessment of these features has shown adequate reliability and specificity (78), but the sensitivity to change over time is relatively small (92).

Quantitative measurements using computer-aided image processing allow for assessment of the whole joint. Direct quantification of cartilage volume, bone surface area, cartilage thickness, and bone marrow lesion area and volume are possible (78). Cartilage morphology assessments are the most widely used quantitative measurement. The measurement of cartilage volume from MRI has been shown to correlate well with the ex-vivo assessments of cartilage volume (stripped away from the bone) (93-96). The accurate segmentation of cartilage from surrounding tissue is an important aspect of assessing cartilage on MRI. The ideal segmentation is one which produces accurate and precise results in a reasonable timeframe.

Despite the growing body of MRI literature, there has been little consistency in its diagnostic application (97). There is a lack of clarity about diagnostic performance and little standardisation regarding MRI interpretation (97). There are also cost concerns compared to plain radiography (97). The lack of uniformity to date is largely due to the absence of a MRI structural definition of OA. Work is currently underway to develop a MRI definition of knee OA and to evaluate MRI as a useful tool in detecting the effects of potential disease-modifying interventions more quickly than is possible with plain

radiographs (97). Using a modified Delphi approach, a panel of experts in the field have developed 11 propositions for a definition of OA on MRI (97). The goal of this exercise was to develop definitions of OA which can be more formally tested in relation to their diagnostic performance.

1.3 Subchondral bone and osteoarthritis

OA was initially considered a disorder of the articular cartilage; however, it is now recognised that OA involves many joint structures including subchondral bone, ligaments, menisci, periarticular muscles, peripheral nerves, and synovium (5). Radin and Rose (98) hypothesised many years ago that subchondral bone plays an important role in OA pathogenesis and there is emerging evidence to support this. This thesis examines the role that subchondral bone plays in OA using data from a prospective population-based study of community-dwelling older adults. It focuses on different subchondral bone measures, such as bone mineral density (BMD), bone size, and bone marrow lesions (BMLs), and how these measures relate to disease progression and disease severity. A comprehensive introduction specific to each research question will be presented at the start of Chapters 4-8. Therefore, this section presents an overview of how subchondral bone relates to OA and provides a rationale for examining the aforementioned subchondral bone measures.

1.3.1 Definition

In general the subchondral bone refers to the bone directly beneath the articular cartilage. Figure 1.2 demonstrates the different layers of cartilage and subchondral bone. The subchondral bone plate consists of cortical bone and is separated from the articular cartilage by the zone of calcified cartilage. The subarticular spongiosa lies beneath the subchondral bone plate and consists of trabecular bone. The subchondral bone plate and the subarticular spongiosa make up the subchondral bone.

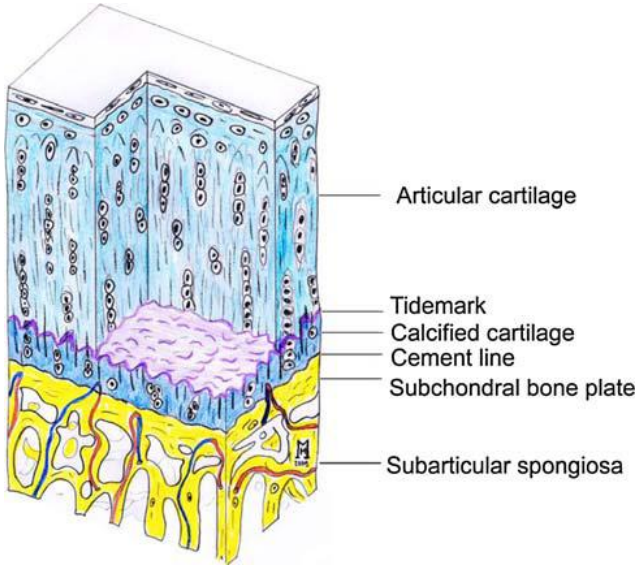


Figure 1.2. Schematic drawing of the different layers of cartilage and subchondral bone (99), with permission.

1.3.2 Function

One of subchondral bone's primary roles is to provide support to the overlying articular cartilage (100). It is believed that the subchondral bone absorbs most of the mechanical force transmitted through the joint (98, 101, 102) and its architecture can adapt during mechanical stress (103). The subchondral bone also plays an important role in articular cartilage metabolism. Canals exist in the subchondral bone which allow blood vessels to reach the cartilage to provide nutrients (99).

1.3.3 Subchondral bone changes in osteoarthritis

Subchondral bone properties are modified through the cell-mediated process of remodelling and modelling (104). Remodelling or bone resorption is mediated by osteoclasts which are responsible for degrading the bone microenvironment (104). Following this is the modelling or bone formation phase in which osteoblasts build up a new extracellular matrix (104). In ideal physiological conditions, the amount of bone removed during the resorption phase is balanced during the formation phase. This cellular process provides a mechanism for repairing damage that occurs to the bone during mechanical loading and stress (105, 106). During the OA process the breakdown and repair phases are disrupted resulting in subchondral bone structure changes. There is a progressive increase in the subchondral plate thickness, a modification in the architecture of subchondral trabecular bone, formation of new bone at the joint margins (osteophytes)

and development of subchondral cysts and lesions (104). The tidemark (displayed in Figure 1.2) is a line of separation between the articular cartilage and calcified cartilage. In OA thickening of the calcified cartilage followed by advancement of the tidemark has been observed in the hand (107) and other large joints such as the hip, shoulder, and knee (108). It is suggested that this may be due to vascular invasion of the calcified cartilage from the subchondral bone (109). Tidemark advancement contributes to the overall thinning of articular cartilage (104).

Radin and Rose (98) originally suggested that bone changes occur very early in the course of OA and this has now been confirmed using numerous study approaches. Animal models have been important for studying the chronological order of OA disease pathology. In a shock-load experiment in rabbits, it was found that increased stiffness of subchondral bone reduced its shock-absorbing ability and induced cartilage degeneration (110). In elderly cynomolgus macaques, thickening of the subchondral bone was shown to occur prior to cartilage destruction and the thickness of the subchondral bone was related to the onset of cartilage fibrillation (111). In a more recent study, Muraoka et al (112) have demonstrated using a spontaneous OA guinea pig model that subchondral bone is fragile before the onset of cartilage degeneration.

Studies in humans have also confirmed that subchondral bone changes occur early on in OA. Studies using isotope-labelled bone-seeking agents in patients with hand OA have found that changes in bone turnover precede bony changes detected by standard radiographs such as osteophytes and sclerosis (113, 114). In a scintigraphy study using technetium-labelled bisphosphonate, Dieppe et al (115) studied patients with established knee OA over a 5 year period. They found that elevated subchondral bone cell activity at baseline predicted subsequent loss of joint space on radiographs. Importantly joint space loss did not occur in those patients with normal bone cell activity at baseline. More recently, numerous studies using MRI have shown that various subchondral bone measures predict disease progression such as quantitative cartilage volume loss. This is covered in more detail in the following sections.

Although it is well recognised that bone changes occur early in OA, whether or not bone changes precede cartilage damage remains controversial. Radin and Rose (98) originally suggested that the health and integrity of the joint cartilage is dependent on the mechanical properties of the underlying subchondral bone. They believed that the initiation of the disease may be partly due to the thickening and increased bone mass of the subchondral bone. This concept has been supported by surgical, mechanical, and spontaneous OA animal models showing that subchondral bone changes precede cartilage

changes (110-112, 116-119). However these studies may not represent the usual mechanism of OA development. Furthermore some animal models demonstrate conflicting results. For example, a shock-load experiment in rabbits found that changes in the mechanical properties of subchondral bone and cartilage degeneration occurred simultaneously (120). Studies conducted in humans with OA have mostly focused on those with late or end-stage disease, which reveals little about sequential order. Regardless of whether bone changes precede cartilage damage, bone does play an important role in OA disease pathology and there appears to be tight coupling between bone and cartilage damage. The following sections will provide an overview of the different subchondral bone measures used in this thesis.

1.4 Bone mineral density and osteoarthritis

It has been suggested that an inverse relationship exists between osteoporosis and OA. This first arose in the 1970s when surgeons noticed that femoral heads resected for treatment of hip OA were much less likely to have evidence of osteoporosis (121). Since then numerous studies have examined the relationship between OA and BMD. Cross-sectional studies have demonstrated that the presence of radiographic knee and hip OA is associated with greater BMD at the lumbar spine, femoral neck, and/or total body (122-127). However, longitudinal studies have revealed a more complex relationship between OA and osteoporosis. In the Chingford Study, Hart et al (128) found that a high BMD at the lumbar spine and hip at baseline was associated with the development of knee osteophytes but not JSN. On the contrary, they also reported that low bone density at the hip was weakly associated with OA progression. In the Study of Osteoporotic Fractures, Arden et al (129) found that radiographic hip OA was associated with reduced bone loss in the femoral neck compared with controls over approximately 7 years. However, radiographic hip OA showed non-significant trends towards increased bone loss at the calcaneus and lumbar spine. Additionally, despite a positive association between BMD and OA, participants with OA did not have a significantly reduced risk of osteoporotic fracture. In the Framingham Study, Zhang et al (130) reported that a high femoral neck BMD and femoral neck BMD gain was associated with incident knee OA. However, they also found that among women with knee OA, low femoral neck BMD and bone loss was associated with increased radiographic OA progression. In a recent study from the Multicentre Osteoarthritis Study (MOST), Nevitt et al (131) reported that a higher systemic BMD is

associated with the onset of JSN in knees without ROA at baseline. In contrast to previous studies (128, 130), they did not find an association between progression and BMD.

In summary the evidence for an inverse association between OA and osteoporosis mainly comes from cross-sectional studies and is not widely supported by longitudinal studies. The relationship between the two diseases appears to be more complex. A high BMD is associated with the development of incident knee OA; however, studies examining BMD and progression have been inconsistent. This may be because the relationship between BMD and OA is explored using radiographs which have been taken at sites different to where BMD is measured. There is now increasing interest to study bone density at the site of OA joints.

1.4.1 Subchondral bone mineral density

Our group, as well as others, have shown that dual-energy x-ray absorptiometry (DXA) applied to the subchondral bone of the tibia is a reproducible and valid technique in measuring subchondral bone mineral density (sBMD) (132-134). Cross-sectional studies have produced conflicting data as to whether the density of the subchondral bone is elevated in OA. Karvonen et al (135) reported lower than normal BMD in the subchondral regions of patients with knee OA. Conversely, other studies have demonstrated that an elevated sBMD is associated with ROA; including osteophyte formation, JSN, and subchondral sclerosis (132, 136). Lo et al (137) found that BMLs occur in knees with relatively higher local tibial bone density. In a separate study Lo et al (138) reported that meniscal damage is associated with higher regional tibial BMD in the same compartment. There are a number of reasons why these differences may exist. First, ROI placement and size varies from study to study and site-specific tibial BMD differences may exist. Second, measurement techniques for assessing tibial BMD are variable; for example, Lo et al (137) used a BMD ratio (between the medial and lateral compartment). Third, the definition of knee OA varies in each study as different outcome factors were assessed. Lastly, the cross-sectional nature of these studies precludes any inference about cause and effect relationships. Longitudinal studies examining sBMD and OA are lacking. Bruyere et al (133) found that those patients with the highest tibial sBMD at baseline experienced the most JSN after 1 year. This lends support to the hypothesis that an elevated sBMD may lead to cartilage damage; however, this needs confirmation by MRI studies.

1.5 Tibial bone size and osteoarthritis

Tibial subchondral bone area is related to OA and can be measured using MRI with high reproducibility (139, 140). In a cross-sectional study, Jones et al (88) showed that those with grade 1 osteophytes had increased medial and lateral tibial plateau bone area compared with those with no evidence of OA. Those with a higher grade of osteophytes were not included in the study and no association was seen between JSN and bone area. In a separate study in women only, Wluka et al (141) found that bone area was associated with both osteophytes and JSN, reporting that increasing severity of ROA was associated with larger tibial plateau bone area.

Longitudinal studies have also demonstrated a relationship between bone area and OA. In subjects with OA, Wang et al (142) found that increasing grade of medial JSN was positively associated with the annual percentage increase of medial tibial plateau area. They reported that medial and lateral tibial bone areas increased by 2.2% and 1.5% per annum over 2 years (142); compared to a healthy cohort of women whose medial and lateral tibial plateau area increased by 1.2% and 0.8% per annum over 2.5 years, respectively (143).

Studies have now shown an association between bone area and cartilage defect development measured using MRI. Ding et al (139) found that an elevated tibial bone area predicted cartilage defect development over time in younger, healthy individuals. Davies-Tuck et al (144) confirmed that an elevated tibial bone area is a risk factor for defect progression in those with well-established symptomatic knee OA. These studies suggest that increased knee bone size is causally related to knee cartilage defects. However, it is unknown whether this is due to increases in bone size, bone density, or both. Cartilage defects are also described as a major contributor to cartilage volume loss (145, 146); thus, it is reasonable to hypothesise that an elevated tibial bone area will also predict cartilage volume loss; although studies have failed to demonstrate this to date (147-149).

1.6 Bone marrow lesions and osteoarthritis

Bone marrow lesions (BMLs) have been recognised as an important feature in knee OA (150, 151). They are detected on MRI and are characterised as non-cystic ill-defined subchondral areas of high signal intensity on T2-weighted or proton density-weighted fat suppressed (FS) fast spin echo (FSE) or short tau inversion recovery (STIR) images (152, 153). BMLs were initially termed ‘bone marrow edema’ by Wilson et al (154) who identified them in patients with severe knee and hip pain. Since then histological studies

have found that actual ‘edema’ is not a major feature and they were renamed ‘bone marrow lesions’. A histological study by Zanetti et al (152) found that BMLs in knees in subjects with severe OA undergoing total knee replacement consisted of several abnormalities including bone marrow necrosis (11%), abnormal trabeculae (8%), bone marrow fibrosis (4%), bone marrow edema (4%), and bone marrow bleeding (2%). Additional MRI-histologic correlation studies have demonstrated fat cell destruction and fibrovascular regeneration in the lesion area (155), as well bone marrow fibrosis in well-defined subchondral zones of OA (156). Recently Hunter et al (157) demonstrated that BMLs are sclerotic compared with unaffected regions from the same individual based on the increased bone volume fraction and increased trabecular thickness.

It has been suggested that BMLs may be an early marker for disease progression. In patients with knee OA Felson et al (151) found that BMLs were associated with joint space loss on radiographs. Subsequent studies have shown that BMLs predict cartilage defect progression (158-161) and cartilage loss (158, 159, 162-164) on MR images. Increasing or progressive BMLs are also associated with increased cartilage loss (165-167). Despite the fact that BMLs predict cartilage damage, it still remains unclear whether BMLs precede, accompany, or follow cartilage damage and volume loss in OA (164).

Despite how common knee pain is, much remains unknown about the nature, causes, and natural history of OA pain (13). Cartilage is the principal structure involved in OA; however, it possesses few pain-sensitive fibres (13). Potential sources of pain include stretching of periosteum from osteophyte growth, raised intraosseous pressure, microfractures, ligament damage, capsular tension, meniscal injury, anserine bursitis and synovitis/effusions (13, 168-170). There appears to be a large heterogeneity of pain in OA. Patterns of disease can be identified using location of pain (171), precipitating and relieving factors (172), and response to intravenous local anaesthetic (173). By identifying the exact causes of pain it would provide the opportunities for a more tailored approach to pain management (13).

There is increasing evidence linking BMLs to knee pain. In a cross-sectional study in patients with knee OA, Felson et al (150) found that BMLs were associated with the presence of knee pain. Zhai et al (174) confirmed that BMLs were cross-sectionally associated with knee pain in a population-based study of older adults. Longitudinal studies have shown that in pain-free populations, incident BMLs (175) and increases in BMLs (81) are associated with development of knee pain. However, other studies in mostly OA subjects have reported no association between changes in BMLs and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index pain scores at baseline (176),

WOMAC scores after 2 years (177), or changes in WOMAC scores (178). Importantly, it remains unknown whether a reduction or resolution of BMLs is associated with improved knee pain.

There is conflicting data on the natural history of BMLs. Most studies have focused on symptomatic OA populations. One study reported that in those subjects with BMLs, <1% of patients showed a BML decrease over 30 months (165) while, in contrast, another study found that 20% of BMLs decreased over 2 years (177). In subjects with prevalent knee OA or at risk for OA, Roemer et al (166) found that the majority (50%) of pre-existing BMLs decreased in size after 30 months follow up. The conflicting data on the natural history of BMLs may be due to different BML grading systems amongst studies. Also, change beyond measurement error is defined differently in each study. Roemer et al (166) used the Whole-Organ Magnetic Resonance Imaging Score (WORMS) semi-quantitative scale to measure BML size. They developed half grade scores in order to detect BML changes which were smaller than 1-unit. Therefore, BML change was defined as any BML increase or decrease ≥ 0.05. Hunter et al (165) also used WORMS but did not apply the half grade scores. Therefore, a BML change was defined as any BML increase or decrease ≥ 1.

Joint replacement surgery is an important outcome in OA research and can be used as a measure of end-stage OA. It is well-established that radiographic severity and pain are strong predictors of joint replacement surgery (179, 180); however, there have been limited prospective studies examining structural factors and knee replacement surgery. In subjects with symptomatic knee OA, ultrasound detected effusion (181), articular cartilage defects (182), rate of tibial cartilage loss and tibial bone size (183) predicted knee joint replacement. A recent study by Tanamas et al (184) showed that the severity of BMLs was positively associated with the risk of knee joint replacement in subjects with well-established OA. Well-established OA in this study was defined as knee symptoms (at least one pain dimension of the WOMAC index score >20% and osteophytes present); and radiographic knee OA (American College of Rheumatology (ACR) radiographic criteria (63)). It is currently unknown whether BMLs in a community-based sample also predict knee joint replacement.

There is growing evidence implicating nutritional factors in OA (185). Specifically, nutrient and dietary supplements have been shown to be effective in relieving OA symptoms and some may play a role in the course of the disease (186). Elevated levels of fat and n-6 polyunsaturated fatty acids have been found in OA bone (187); whereas n-3 polyunsaturated fatty acids have been shown to modulate catabolic factors in articular

cartilage destruction (188). There is limited data on the relationship between fatty acids and BMLs. Wang et al (189) reported that higher intakes of monounsaturated, total, and n-6 polyunsaturated fatty acids were associated with BMLs cross-sectionally. In a recent longitudinal design, they showed that increased saturated fat intake was associated with incident BMLs (190). These results need confirmation in different settings. Furthermore, it is unknown whether intakes of other dietary components such as total energy, protein, carbohydrate, and/or sugar are associated with BMLs.

Research has shown that the prevalence of vascular disease is high amongst people with OA (187, 191). Evidence suggests that these diseases may share risk factors, such as obesity, hypertension, high low-density lipoprotein (LDL) levels, elevated total cholesterol, diabetes, smoking, and diet (187, 191-194). Vascular pathology may contribute to the development of OA through its effects on the subchondral bone. Blood flow through the small vessels in the subchondral bone may be reduced by venous occlusion, which results in impaired venous circulation underlying the cartilage plate, joint hypertension, hypercoagulability and/or microemboli (192). These may result in subchondral bone ischemia, which can contribute to decreased nutrient supply to the overlying cartilage plate (192). Subchondral bone ischemia can also affect osteocyte death, leading to bone resorption, reducing the viability of subchondral bone (192, 195). As previously outlined, BML histology is heterogeneous and includes osteonecrosis, edema, trabecular abnormalities and bone remodelling (152). Additional MRI-histologic correlation studies of these lesions have demonstrated fat cell destruction and fibrovascular regeneration in the lesion area (155), as well bone marrow fibrosis in well-defined subchondral zones of OA (156). Recently Hunter et al (157) demonstrated that BMLs are sclerotic compared with unaffected regions from the same individual based on the increased bone volume fraction and increased trabecular thickness. Also, BMLs have been linked to ischemia and/or reperfusion injury (195, 196). Therefore it is possible that vascular pathology may influence BML development. To our knowledge there has been only one study which examined serum lipids and BMLs, reporting that serum cholesterol and triglyceride levels were associated with an increased incidence of BMLs (197). However, this study was conducted in asymptomatic women; therefore, further studies are needed in different populations to confirm this finding. Additionally is unknown whether serum lipids are associated with BML progression.

In summary BMLs are an important feature in OA and further investigation is needed to understand their role in disease pathogenesis. Preliminary studies suggest a systemic role in BML pathology. The role diet and blood lipids play in BML development

should be investigated further to better understand the mechanism behind these associations.

1.7 Summary

By 2050, it is projected there will be 3.1 million Australians or 11% of the population with OA. OA inflicts a major economic and social burden on society. Until recently, cartilage degeneration was considered the initial pathologic defect in OA. It is now recognised that many other joint structures are involved. Subchondral bone refers to the bone directly beneath the articular cartilage. It plays an important role in biomechanical and biochemical functions in the joint. Subchondral bone changes are thought to occur at early stages of disease. The following chapters investigate the role subchondral bone plays in knee OA using data from a population-based study of community-dwelling older adults. Novel analyses have been performed examining the association of sBMD, bone size, and BMLs with measures of cartilage defect development, cartilage volume loss, pain, knee replacement surgery, dietary intake, and serum lipids. The research questions which directed this work are described in the following chapter.

Chapter 2 - Research questions

2.1 Research Questions

In a population-based sample of community-dwelling adults aged 50–80 years examined at baseline and approximately 3 years later:

1) What is the cross-sectional association between tibial subchondral bone mineral density (sBMD) and anthropometric, environmental, lifestyle, and structural measures?
2) What is the association between baseline tibial bone area and tibial sBMD with tibial cartilage defect development and cartilage volume loss?
3) Does baseline bone marrow lesion (BML) presence and/or severity predict site-specific changes in cartilage (defects and/or volume changes); and does baseline cartilage defect presence and/or severity predict site-specific BML progression?
4) What is the natural history of BMLs and are BMLs associated with pain, function, and stiffness scores, and total knee replacement surgery?
5) What is the association between dietary factors, serum lipids, and BMLs?

2.2 Key Hypothesis

Subchondral bone measures including sBMD, tibial bone area, and BMLs will be associated with disease progression in a community-based sample of older adults. Specifically:

1) Denser subchondral bone and larger tibial bone size will lead to cartilage damage.
2) BMLs will be associated with pain and predict cartilage damage and loss.

Chapter 3 - Methodology

3.1 Prelude

This thesis arose from analyses of the Tasmanian Older Adult Cohort (TASOAC) study population, and a number of outcome factors, study factors, and covariates have been utilised. This chapter describes the TASOAC study population and its design, as well as the protocols for measurement of factors which are common to multiple chapters in this thesis. Additional factors which are unique to each chapter are described in more detail within the methodology section of each of the subsequent chapters.

It should be noted that the following chapters are presented in the form in which they were submitted to, or accepted by, peer-reviewed journals for publication. Thus, throughout these chapters there are some differences in the description of methods, analyses, results, and interpretations, due chiefly to requests from journal reviewers.

3.2 Study population and design

The work in this thesis was conducted as part of the TASOAC study, an ongoing prospective, population-based study aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of osteoarthritis (OA) at multiple sites (hand, knee, hip, and spine). The cohort consisted of both males and females aged between 50 and 80 years (mean ± standard deviation (SD) age = 62 ± 7 years), selected from the roll of electors in southern Tasmania (population 229,000) using stratified simple random sampling without replacement. Electoral rolls represent the most complete population information available in Australia because voting in federal and state elections is compulsory. The sample was stratified by sex to provide equal numbers of men and women, and equal distribution was drawn from urban and rural areas in southern Tasmania. As TASOAC was designed to examine community-dwelling older adults, institutionalised older adults were excluded. Participants were also excluded if they reported contraindication for magnetic resonance imaging (MRI), as these tests were required to examine OA progression.

Figure 3.1 provides an overview of participant recruitment and withdrawal during the study period. 2,135 initially eligible participants were identified from which 1,904 were able to be contacted. Of all initially eligible participants, 1,100 enrolled in the study, and 1,099 attended a baseline clinic between March 2002 and September 2004 (response fraction 51%). Follow-up data was collected for 875 eligible participants (80%) at a subsequent clinic approximately 2–3 years later (mean ± SD 2.7 ± 0.4; range: 1.4-4.8 years). Table 3.1 summarises the baseline demographic characteristics of those participants

who completed the follow-up (n=875) and those which did not (n=224). Those participants who completed the follow-up were younger, taller, and had a lower BMI compared to those who did not complete the follow-up. There were also a higher proportion of men in those which completed the follow-up.

The MRI machine was decommissioned halfway through the follow-up period; therefore, MRI scans were only available for approximately half of the follow-up participants (n=425/875). Table 3.2 summarises the demographic and clinical follow-up characteristics of those participants who had an MRI scan at follow-up (n=425) and those which did not (n=450). There were no significant differences between those who had an MRI scan at follow-up and those which did not.

The sample size used in Chapters 4–8 of this thesis varies depending on the available data for each of the research questions.

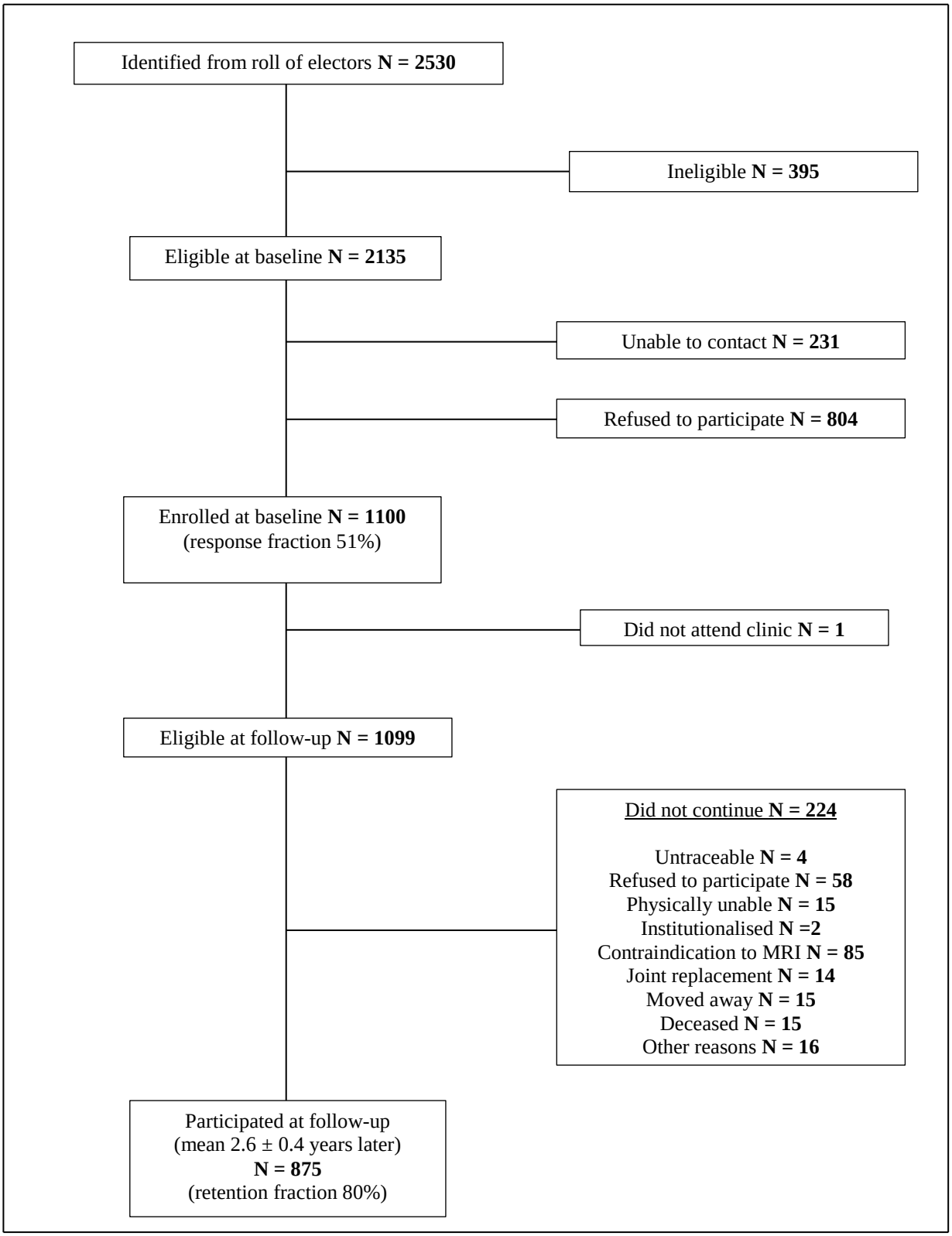


Figure 3.1. Flow chart describing recruitment, participation rates, and withdrawal reasons for TASOAC participants.

Table 3.1. Baseline demographic characteristics of those participants who completed the follow-up (n=875) and those which did not (n=224)

	Participated in the follow-up (n=875)	Did not continue (n=224)	*P* values
Age (year)	62.7 (7.3)	64.4 (8.2)	<0.01
Male sex (%)	51	41	<0.01
Height (cm)	167.5 (9.0)	164.9 (8.8)	<0.01
Weight (kg)	77.9 (14.6)	77.7 (16.5)	0.824
BMI (kg/m2)	27.7 (4.6)	28.5 (5.7)	0.040

*Mean (standard deviation) except for percentages. *P* values determined by t-test or chi-square test (where appropriate). BMI: body mass index.

Table 3.2. Demographic and clinical follow-up characteristics of those participants who had an MRI scan at follow-up (n=425) and those which did not (n=450)

	MRI scan at follow-up (n=425)	No MRI scan at follow-up (n=450)	*P* values
Age (year)	65.7 (7.2)	64.8 (7.4)	0.079
Male sex (%)	49	52	0.406
Height (cm)	166.8 (9.0)	167.3 (9.1)	0.406
Weight (kg)	77.5 (14.4)	78.7 (15.2)	0.247
BMI (kg/m2)	27.8 (4.6)	28.1 (4.9)	0.432
WOMAC			
Pain (0–45)	2.9 (6.0)	2.6 (4.6)	0.368
Function deficit (0–153)	8.8 (18.4)	8.7 (16.5)	0.923
Stiffness (0–18)	1.9 (2.7)	1.3 (2.3)	0.797
Pain knees, yes/no (% yes)	38	40	0.565

*Mean (standard deviation) except for percentages. *P* values determined by t-test or chi-square test (where appropriate). BMI: body mass index; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

3.3 Anthropometrics

Each subject's body weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford, MA, USA). Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kg/m^2.

3.4 Dual energy x-ray absorptiometry (DXA)

Bone mineral density (g/cm^2) was measured at the tibia using a Hologic Delphi densitometer (Waltham, MA, USA). Participants were in a supine position, and the knee was in full extension. Subchondral bone was defined as the entire tibia proximal to the head of the fibula, which is in line with recent reports (136). Medial and lateral subchondral bone mineral density (sBMD) of the tibia was measured using existing spine software. Three separate regions of interest (ROIs) were used based on our previous work on the reproducibility and validity of measuring sBMD using dual energy x-ray absorptiometry (DXA) (134). The three ROIs for each compartment were drawn manually around the bone and are displayed in Figure 3.2 (A–C). The three ROIs refer to methods 2, 3, and 4 in our previous pilot study where we demonstrated reproducibility and validity (134) (Appendix 1).

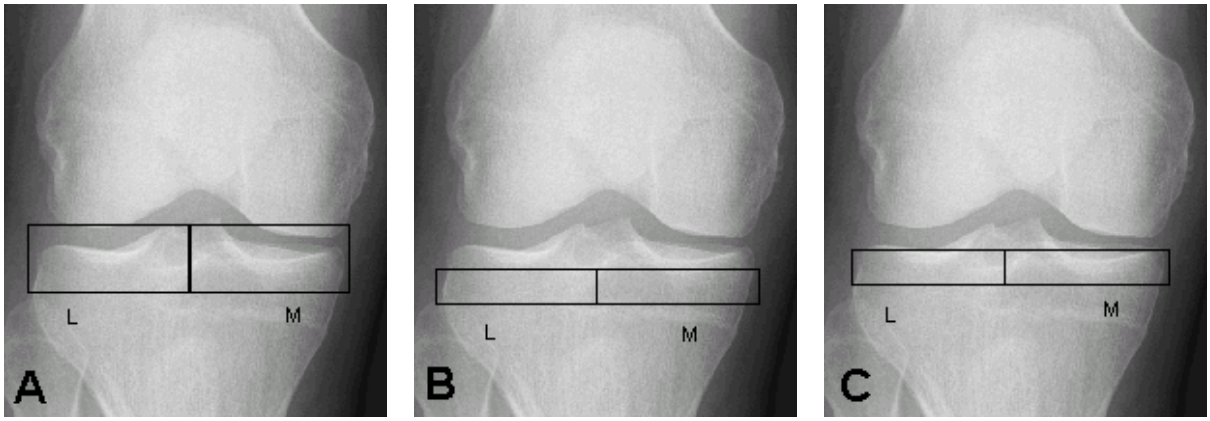


Figure 3.2. Three (A–C) medial and lateral ROIs used to measure sBMD.

In Figure 3.2, ROI 1 (A) used the midpoint of the two intercondylar spines as a reference; the top of the ROI was the highest point of the medial or lateral spine and extended to the edge of the image, either medially or laterally. It was a height of 20 mm and the width of the tibial bone. This ROI included either the medial or lateral intercondylar spine. If the ROI included the femur due to a tall intercondylar spine, the

ROI was moved down accordingly to exclude the femur from the measurement. ROI 2 (B) used the midpoint of the two intercondylar spines as a reference and descended 10 mm down from the highest point of the medial or lateral spine. The ROI extended to the edge of the image, either medially or laterally. The ROI was a height of 10 mm (10 to 20 mm beneath the top of the tibial spine) and the width of the tibial bone. ROI 3 (C) used the top of the tibial surface as a reference and was a height of 10 mm. The width of the ROI extended to the edge of the image, either medially or laterally.

Tibial sBMD was measured in 740 of the 1099 TASOAC participants. DXA measurements were not available for 359 participants because a disk containing their scan was misplaced during a building move. No significant demographic differences existed between those participants whose DXA scans were misplaced and those with complete DXA scan results.

3.5 Magnetic Resonance Imaging

MRI of the right knee was acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit/receive extremity coil. Image sequences included the following: (1) a T1-weighted fat saturation three-dimensional (3D) gradient-recalled acquisition in the steady state, flip angle 30º, repetition time 31 ms, echo time 6.71 ms, field of view 16 cm, 60 partitions, 512 × 512–pixel matrix, acquisition time 5 min 58 s, one acquisition; sagittal images were obtained at a slice thickness of 1.5 mm without a interslice gap; and (2) a T2-weighted fat saturation two-dimensional (2D) fast spin echo, flip angle 90º, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228 × 256–pixel matrix; sagittal images were obtained at a slice thickness of 4 mm with a interslice gap of 0.5–1.0 mm.

These sequences were chosen to image cartilage, bone and meniscus. The T1-weighted sequence was chosen for optimal assessment of cartilage defects, cartilage volume, tibial bone area, and meniscus. The T2-weighted sequence was chosen to assess bone marrow lesions (BMLs). These are described in further detail below.

3.5.1 Tibial bone area

Knee tibial plateau bone area was assessed on T1-weighted MR images and defined as the cross-sectional surface area of the tibial plateau, as previously described (88, 139, 198). Medial and lateral bone area was measured manually by reformatting the whole sagittal image to the axial plane. Area was then measured on three slices closest to the

tibial cartilage and the mean of all three areas was used as an estimate of tibial plateau bone area. The slice thickness on the axial images was 0.625 mm. In a previous study, the coefficient of variation (CV) in our hands for this method of measurement was 2.2–2.6% (88).

3.5.2 Bone marrow lesions

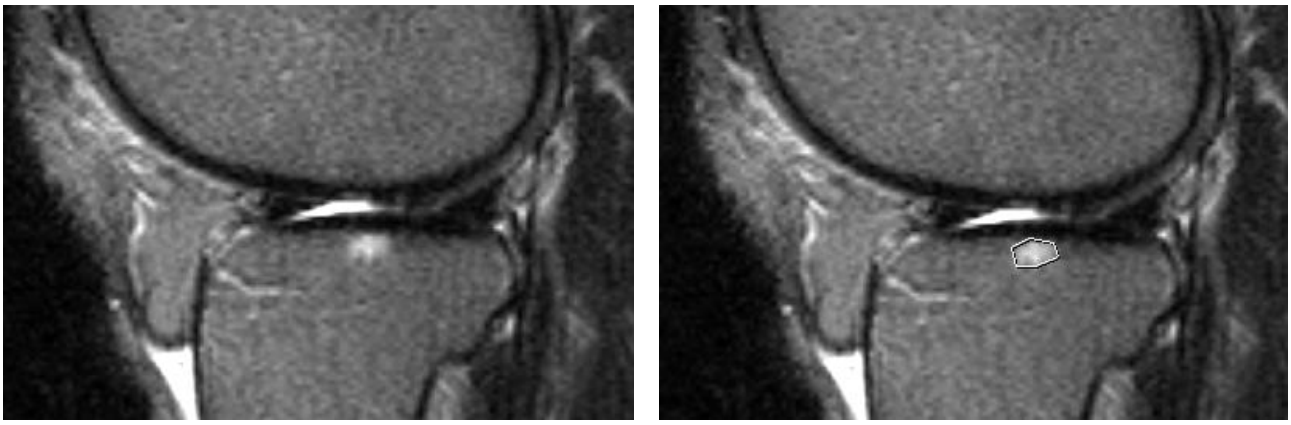
Subchondral BMLs were assessed on T2-weighted MR images using Osiris software (University of Geneva, Geneva, Switzerland) and were defined as areas of increased signal adjacent to the subcortical bone at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites. If a lesion crossed these anatomical sites, it was scored twice. For example if a BML was large enough to cover both the medial and lateral femur, it would be scored two times, once as a medial femoral BML and twice as a lateral femoral BML. This may introduce measurement error; however, when evaluating site-specific associations it is important to capture every anatomical site. BMLs were scored using both an ordinal scoring system and an areal scoring system.

Ordinal scoring system for BMLs

Each BML was scored on the basis of lesion size (e.g. a lesion was scored as grade 1 if it was only present on one slice, grade 2 if present on two consecutive slices, or grade 3 if present on three or more consecutive slices). The BML with the highest score was used if more than one lesion was present at the same site. Intraobserver repeatability was assessed in 50 subjects with at least a one week interval between the two readings. The intraclass correlation coefficients (ICCs) were 0.94, 1.00, 0.89, and 0.96 at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, respectively.

Areal scoring system for BMLs

Each BML was also scored by measuring the maximum area of the lesion as demonstrated in Figure 3.3. The observer manually selected the MRI slice with the greatest BML size. The BML with the highest score was used if more than one lesion was present at the same site. The maximum area was measured in mm^2 using software cursors. Intraobserver repeatability was assessed in 40 subjects with at least a two week interval between the two readings. The ICC was 0.97. Participants were given a BML score (mm^2) at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites.



(A) (B)

Figure 3.3 (A) Lateral tibial BML. (B) Areal measurement of the BML (15 mm^2).

3.5.3 Cartilage defects

Cartilage defects were assessed on T1-weighted MR images (score range, 0–4) at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as previously described (199) as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness <50%; grade 3 = deep ulceration with loss of thickness >50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. A cartilage defect also had to be present on at least two consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. The highest score was used if more than one defect was present on the same site. One observer scored the cartilage defects. Intraobserver repeatability was assessed in 50 subjects with an interval of at least one week between the two measurements. ICCs were 0.93, 0.92, 0.95, and 0.80 at the medial tibia, medial femur, lateral tibia, and lateral femur, respectively.

3.5.4 Cartilage volume

Knee tibial cartilage volume was assessed on T1-weighted MR images by means of image processing on an independent workstation using Osiris (University of Geneva, Geneva, Switzerland) software as previously described (140, 200). The volumes of individual cartilage plates (medial tibia and lateral tibia) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a

section by section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312 × 312 mm and 1.5 mm thickness, continuous sections) for the final 3D rendering. The CV in our hands for this method of measurement was 2.1% for the medial tibia and 2.2% for the lateral tibia (200).

3.6 X-ray

A standing anteroposterior semiflexed view of the right knee with 15° of fixed knee flexion was performed. Radiographs were assessed using the atlas developed by Altman et al (77). Each of the following was assessed on a scale of 0–3: medial joint space narrowing (JSN), lateral JSN, medial femoral osteophytes, medial tibial osteophytes, lateral femoral osteophytes, and lateral tibial osteophytes. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. Intraobserver repeatability was assessed in 40 subjects with an interval of at least one week between the two measurements. ICCs ranged from 0.65–0.85. The presence of radiographic osteoarthritis (ROA) was defined as any score ≥1 for JSN or osteophytes.

3.7 Physical activity measures

Physical activity was assessed as steps/day determined by pedometer (Omron HJ-003 & HJ-102, Omron Healthcare, Kyoto, Japan). Pedometers were calibrated at the clinic with the participants present, using a 100-pace walking test. All participants were given verbal and written instructions regarding pedometer wear and how to keep a pedometer diary (demonstrated in Appendix 2). Each participant wore the pedometer for seven consecutive days and recorded the number of steps they did each day and the duration and type of physical activity for any activities in which the pedometer could not be worn (e.g. swimming). An average of the seven days was used to give each participant a mean steps/day value. Participants were then mailed out a second pedometer after a six month period and repeated the process. A steps/day value, which was an average of their steps/day in summer and winter, was calculated. Pedometer readings were excluded if they were determined to be caused artificially (e.g. report of work done on heavy machinery), or if the pedometer had been worn for less than five days. Pedometer determined physical activity is a good reflection of usual physical activity (201, 202) and is more accurate than self-reported walking behaviour (203). Piezoelectric pedometers, such as the ones used in this study, have been shown to be more accurate than spring-levered pedometers (e.g., Yamax SW-200) at the slower walking speeds typical among older adults (204). Melanson

et al (204) reported that piezoelectric pedometer s detected 97.8 ± 9.6% of counted steps at 1.8 miles per hour (MPH) and 56.4 ± 33.8% of counted steps at 1.0 MPH.

Leg strength was measured by a dynamometer (TTM Muscular Meter; Gloria, Tokyo, Japan) with both legs involved simultaneously. The muscles measured with this technique are predominantly quadriceps and hip flexors. Subjects were instructed in the technique prior to testing. Each subject had two attempts and an average of the two was taken. Repeatability estimate (Cronbach's alpha) was 0.91.

3.8 Summary of outcome factors, study factors, and covariates

Table 3.3 summarises the variables used in each chapter of this thesis.

Table 3.3. Summary of outcome factors, study factors, and covariates used in this thesis

Chapter	Outcome factors	Study factors	Covariates
4	sBMD	Age, sex, height, weight, BMI, 25(OH)D)*, sun exposure*, steps/day, leg strength, smoking*, alcohol consumption*, osteophytes, JSN, BMLs, cartilage defects, cartilage volume, bone area, spine and hip BMD*	Age, sex, BMI
5	Cartilage defects Cartilage volume	sBMD, bone area	Age, sex, BMI
6	Cartilage defects Cartilage volume BMLs	BMLs Cartilage defects	Age, sex, BMI, meniscal extrusion*, meniscal tear*, ROA
7	Pain* Function* Stiffness* TKR*	BMLs	Age, sex, BMI, leg strength, quality of life*, cartilage defects, bone area, ROA
8	BMLs	Dietary intake* (energy, fat, carbohydrate, protein, and sugar) Serum lipids* (total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol	Age, sex, BMI, smoking*, cardiovascular disease*, diabetes*, ROA, statin use*

*Measurement protocol described in "Materials and Methods" section of relevant chapter.

3.9 Sample size and role of the candidate in the TASOAC study

As the TASOAC study was in progress before commencement of the PhD candidature formal sample size calculations were not performed during the design of this thesis. Therefore, participant numbers in the analyses reported in this thesis were limited to the numbers recruited at baseline and follow-up, and to those who provided complete data for relevant outcome and study factors. As such, sample sizes vary between chapters, and the reasons for exclusion are described in each chapter. Nevertheless, it subsequently proved that sample sizes were more than adequate to answer the thesis research questions as this thesis has reported significant findings related to the research questions.

The candidate was involved in TASOAC data acquisition and collection, data management, analysis and interpretation of data, initial manuscript preparation and manuscript revision. Data acquisition was also completed prior to and during the candidature by a number of other TASOAC staff and volunteers, including Graeme Jones, Changhai Ding, Catrina Boon, Dale Pitt, Bronwyn Archer, Pam McDonald, Stella Foley, and David Scott. Several colleagues had also begun analyses using TASOAC data before candidature was undertaken, and the candidate gratefully acknowledges the assistance of Changhai Ding in cleaning the cartilage volume, cartilage defect, and bone area data; Jenny Cochrane and Stella Just in cleaning of pedometer data; and Jenny Cochrane in cleaning the dietary data.

3.10 Ethical considerations

All procedures in TASOAC were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee (Ethics Approval Number: H6488). Written informed consent was obtained from all participants prior to enrolment in the study.

3.11 Statistical analysis

T-tests and chi-squared tests were used to compare differences in means and proportions as appropriate. A *P* value less than 0.05 (two-tailed) was considered statistically significant. A more detailed description of statistical analyses performed are presented in their relevant chapters. All statistical analyses were performed on Intercooled Stata versions 8, 9 or 10 for Windows (StataCorp, College Station, TX, USA).

Chapter 4 - Correlates of subchondral bone mineral density: a cross-sectional study

4.1 Introduction

Research has shown that conventional radiographic imaging of bone in osteoarthritis (OA) is unsatisfactory for clinical and research purposes (85, 205). Joint space narrowing (JSN) is one of the main features measured on radiographs and can be influenced by many joint structures including cartilage and meniscus. Changes in joint space width (JSW) occur in the later stages of the disease and therefore does not allow for early detection, which eliminates the potential for early therapeutic intervention (132). Additionally, progression of radiographic osteoarthritis (ROA) is slow and varies greatly between individuals (206-208). Plain radiographs are insensitive to JSW changes and are uninformative at less than 2 year intervals (132); therefore, proving to be unreliable for the assessment of OA progression. Magnetic resonance imaging (MRI) can accurately assess cartilage and bone changes; however, it is relatively expensive and not widely available, making MRI unrealistic for assessment of OA in the general population. Therefore, there is great need for a simple, non-invasive, reproducible method to assess joints over time.

Dual energy x-ray absorptiometry (DXA) is a safe, rapid, reliable and inexpensive tool for the assessment of bone mineral density (BMD). Its application in OA is relatively new. Various features of subchondral bone including size and density are involved in the osteoarthritic process (98, 132, 133, 137, 209). Our group, as well as others, have shown that DXA applied to the subchondral bone of the tibia is a reproducible and valid technique in measuring subchondral bone mineral density (sBMD) (132-134). Cross-sectional studies have demonstrated that an elevated sBMD is associated with ROA; including osteophyte formation and JSN, and subchondral sclerosis and bone marrow lesions (BMLs) (132, 136, 137). However, there is a lack of knowledge about the determinants of sBMD and associations with knee structural features. Therefore, the aim of this cross-sectional study was to generate hypotheses regarding the association between sBMD and anthropometric, environmental, lifestyle, and structural measures in a large randomly selected population.

4.2 Materials and Methods

4.2.1 Cartilage defects

Cartilage defects were assessed as described in section 3.5.3. Prevalent cartilage defects were defined as a cartilage defect score of ≥2 at either the tibial or femoral site. For analysis, medial tibial and femoral cartilage defect scores were combined; therefore, medial tibiofemoral cartilage defect scores ranged from 0–8. This was also done for lateral tibiofemoral cartilage defects.

4.2.2 Bone marrow lesions

BMLs were assessed using the ordinal scoring system as described in section 3.5.2.

4.2.3 Serum 25-hydroxyvitamin D

25-hydroxyvitamin D [25(OH)D] was assayed using a Liquid Phase radioimmunoassay (Immunodiagnostics Systems Ltd, Boldon, Tyne & Wear, UK). The intra- and interassay coefficients of variation (CVs) were 1.8% and 3.3%, respectively.

4.2.4 Measures related to sun exposure

Sun exposure was assessed by a standardised questionnaire on the amount of time spent in the sun during weekends and holidays in the past winter and summer (<1, 1–2, 2–3, 3–4, or >4 hr/day). This exposure measure has been validated against actual exposure with polysulphone badges in teenage children in this climate and it was found to correlate well in summer (intraclass correlation coefficient (ICC) 0.62) (210).

4.2.5 Smoking

Smoking status (never, former, and current) was determined by questionnaire from the following questions: “Have you ever smoked cigarettes on a regular basis?”; “If yes, at what age did you start smoking regularly?”; “Do you currently smoke cigarettes?”; and “If you have given up smoking, at what age did you stop?”

4.2.6 Alcohol consumption

Alcohol consumption was assessed by a validated food frequency questionnaire (FFQ) which was developed specifically for use in Australian adults (211). Participants were asked about their average alcohol consumption over the past 12 months. The consumption frequency of each alcohol type [beer (low alcohol); beer (full strength); red wine; white wine (includes sparkling wines); fortified wines, port, sherry, etc.; and spirits, liqueurs, etc.] was asked about separately. An estimated daily intake of alcohol (gram/day) was calculated. An example of the output is provided in Appendix 3.

4.2.7 Bone mineral density

sBMD of the tibia was measured using region of interest (ROI) 1 (A) and 2 (B) as described in section 3.4 and displayed in Figure 3.2. One single scan was analysed for immediate reproducibility and interval reproducibility; therefore, we did not evaluate re-positioning error in this study. sBMD was measured two times consecutively for each ROI and the mean of the two measurements was used in the association's analysis. Immediate reproducibility was calculated as the ICC between these two consecutive measurements. Interval reproducibility was calculated by re-reading 20 of the same scans after a time interval of at least 14 days. One reader completed all of the measurements. The immediate reproducibility was excellent. ICCs for the medial ROI 1 and 2 were 0.96 (95 % CI, 0.94–0.98) and 0.99 (95% CI, 0.99–1.00), respectively. For the lateral ROI 1 and 2, ICCs were 0.95 (95% CI, 0.93–0.97) and 0.97 (95% CI, 0.95–0.98), respectively. After a time interval between measurements, the ICCs were poorer. The ICCs for interval reproducibility for the medial ROI 1 and 2 were 0.91 (95% CI, 0.83–0.99) and 0.52 (95% CI, 0.19–0.84), respectively. For the lateral ROI 1 and 2, ICCs were 0.33 (95% CI, 0.0–0.73) and 0.59 (95% CI, 0.30–0.88), respectively.

BMD was also measured at the hip (neck of the femur) and spine.

4.2.8 Statistical analysis

Standard diagnostic checks of model fit and residuals were made and revealed that the residuals were not normally distributed. Therefore, medial and lateral sBMD using ROI 1 and 2 were transformed to meet model assumptions. Henceforth, we will refer to the transformed sBMD as sBMD*. Partial correlation analysis was used to examine the associations between medial and lateral sBMD* and the anthropometric, lifestyle, and

structural outcome variables. Results are presented as partial correlation coefficients. Interactions were also investigated by partial correlation analysis of product terms (e.g. sex × age) and medial sBMD* after adjusting for confounders.

4.3 Results

4.3.1 Subjects

The current study consists of a consecutive sample of 740 participants (mean age 62 years, range 50–80 years, 52% female) who had their tibial sBMD measured. The characteristics of the study population are presented in Table 4.1. In unadjusted analysis, subjects below the median medial sBMD score were similar to subjects greater or equal to the median score in terms of 25(OH)D level, steps/day, smoking status, and presence of JSN and BMLs. There were a greater proportion of males in the group of subjects above or equal to the median medial sBMD score. Additionally these subjects were younger, taller, weighed more, had a higher body mass index (BMI), reported increased sun exposure, had greater leg strength, had greater levels of alcohol consumption, had a greater prevalence of osteophytes and cartilage defects, had greater cartilage volume and bone area, and had a greater spine and hip BMD.

Table 4.1. Characteristics of participants*

	Medial sBMD < median (n=367)	Medial sBMD ≥ median (n=373)	*P* values
Age (year)	63.2 (7.7)	61.3 (7.3)	<0.001
Male sex (%)	27	69	<0.001
Height (cm)	163.5 (8.3)	170.2 (8.5)	<0.001
Weight (kg)	73.1 (14.5)	82.7 (14.0)	<0.001
BMI (kg/m2)	27.2 (4.6)	28.6 (4.8)	<0.001
25(OH)D (nmol/L)	51.6 (18.4)	52.6 (18.9)	0.474
Summer sun exposure (0–4)	1.6 (1.3)	2.1 (1.5)	<0.001
Winter sun exposure (0–4)	1.4 (1.3)	1.8 (1.4)	<0.001
Steps/day	9112 (3438)	9646 (3867)	0.055
Leg Strength (kg)	73.2 (42.5)	108.6 (49.1)	<0.001
Alcohol consumption (g/day)	13.1 (17.6)	18.5 (21.9)	<0.001
Current Smokers (%)	17	23	0.122
Osteophytes present† (%)	5	10	0.019
JSN present (%)†	53	56	0.377
BMLs present (%)†	19	25	0.095
Prevalent cartilage defects (%)‡	22	29	0.048
Cartilage volume (mL)	2138 (493)	2530 (646)	<0.001
Bone area (mm2)	1837 (267)	2035 (295)	<0.001
Spine BMD (g/cm2)	0.940 (0.161)	1.070 (0.150)	<0.001
Hip BMD (g/cm2)	0.894 (0.136)	1.035 (0.134)	<0.001

*Mean (standard deviation) except for percentages. *P* values determined by t-test or chi-square test (where appropriate). sBMD: subchondral bone mineral density, BMI: body mass index; 25(OH)D: 25-hydroxyvitamin D; JSN: joint space narrowing; BML: bone marrow lesions; BMD: bone mineral density. Structural features represent medial values. sBMD measurements from ROI 1.
Median value of sBMD: 0.115 g/cm2.
† Defined as grade 1 or higher.
‡ Defined as grade 2 or higher.

4.3.2 Anthropometrics

Table 4.2 describes the multivariable relationship between anthropometric factors and medial sBMD*. Medial sBMD* was negatively associated with age and female sex and positively associated with weight and BMI, using both ROIs. The total model explained 25% of the variability of medial sBMD*, of which 21% was attributable to sex (ROI 1). In the unadjusted analysis, medial sBMD* was positively associated with height using both ROIs 1 and 2 (r = 0.37, $P < 0.01$; r = 0.21, $P < 0.01$, respectively). However, in the multivariable analysis adjusting for age and sex, the association did not persist (Table 4.2).

Table 4.2. Multivariable analysis examining the relationship between anthropometric factors and medial sBMD*

	Partial correlation coefficients (r)	
	ROI 1	ROI 2
Age (year)	**-0.20**†	**-0.18**†
Sex (female)	**-0.47**‡	**-0.25**‡
Height (cm)	0.03ϕ	0.02ϕ
Weight (kg)	**0.12**ϕ	**0.17**ϕ
BMI (kg/m^2)	**0.13**ϕ	**0.17**ϕ

sBMD*: transformed subchondral bone mineral density; ROI: region of interest; BMI: body mass index.
Bold denotes a statistically significant result ($P < 0.01$).
†Adjusted for sex and BMI.
‡Adjusted for age and BMI.
ϕAdjusted for age and sex.

4.3.3 Environmental and lifestyle factors

Table 4.3 describes the multivariable relationship between environmental and lifestyle factors and medial sBMD*. After adjustment for age, sex, and BMI, medial sBMD* was positively associated with steps/day using ROI 1. Using ROI 2, medial sBMD* was positively associated with 25(OH)D and winter sun exposure. Figure 4.1 documents the positive association ($P = 0.025$) between medial sBMD and steps/day; after adjustment for age, sex, and BMI (ROI 1). Alcohol was no longer significantly associated with medial sBMD* in the multivariable analysis (Table 4.3).

Table 4.3. Multivariable analysis examining environmental and lifestyle factors associated with medial sBMD*

	Partial correlation coefficients (r)	
	ROI 1	ROI 2
25(OH)D (nmol/L)	0.02	**0.10****
Summer sun exposure (0–4)†	0.03	0.07
Winter sun exposure (0–4)†	0.02	**0.09***
Steps/day	**0.08***	0.07
Leg Strength (kg)	0.04	0.04
Smoking (0–2)‡	-0.02	-0.06
Alcohol consumption (g/day)	0.04	0.06

sBMD*: transformed subchondral bone mineral density; ROI: region of interest.
Adjusted for age, sex, and body mass index.
Bold denotes a statistically significant result (*$P < 0.05$, **$P < 0.01$).
†Sun exposure 0–4: <1 hr/day, 1–2 hr/day, 2–3 hr/day, 3–4 hr/day, or >4 hr/day
‡Smoking 0–2: Never smoked, former smoker, current smoker

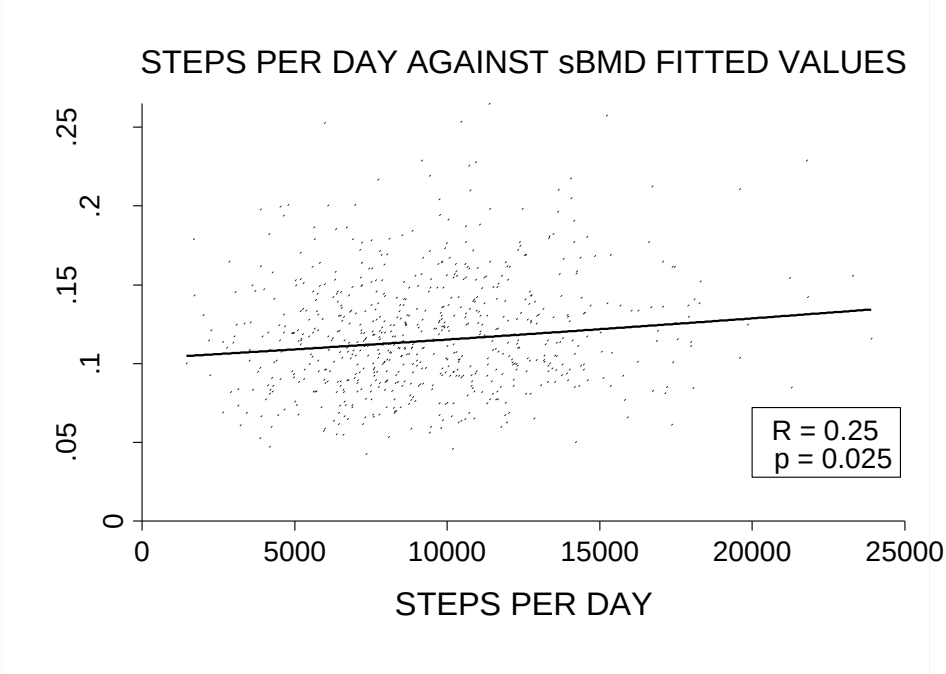


Figure 4.1. Association between medial sBMD using ROI 1 and steps/day. *P* for trend was adjusted for age, sex, and BMI.

4.3.4 Medial sBMD and medial structural measures

Table 4.4 describes the association of medial sBMD* with structural measures. After adjustment for age, sex, and BMI, medial sBMD* was positively associated with tibial osteophytes, JSN, cartilage defects, cartilage volume, tibial and femoral BMLs, and spine and hip BMD, using ROI 1. Using ROI 2, medial sBMD* was positively associated with tibial osteophytes, cartilage defects, tibial and femoral BMLs, and spine and hip BMD. Additionally, cartilage defects remained significantly associated with medial sBMD* after further adjustment for tibial bone area and medial tibial and femoral BMLs using both ROIs 1 and 2 (r = 0.19, $P < 0.01$; r = 0.19, $P < 0.01$, respectively). Using both ROI 1 and 2, medial sBMD* was significantly higher in those subjects who had prevalent cartilage defects ($P = 0.01$; $P < 0.01$, respectively) (Figure 4.2).

For all factors, the best model explained 40% of the variability in medial sBMD* using ROI 1 and 28% using ROI 2.

Figure 4.3 displays the interactions between age and sex, age and BMI, and sex and BMI on medial sBMD*. There was a significant interaction between: (A) age and sex for sBMD* using ROI 1 (there is a negative association between age and sBMD and this appears to be stronger in women compared with men); (B) age and BMI for sBMD* using ROI 2 (there appears to be a steeper increase in sBMD as BMI increases in older adults versus younger age categories); and (C) sex and BMI for sBMD* using ROI 1 (BMI has a positive association with sBMD, and this appears to be stronger in women compared with men).

Figure 4.4 displays the interactions between sex and cartilage volume and sex and BMLs on medial sBMD* using ROI 1. There was a significant interaction between: (A) sex and cartilage volume on sBMD* (cartilage volume is positively associated with sBMD and this appears to be stronger in men compared with women); (B) sex and tibial bone marrow lesions; and (C) sex and femoral bone marrow lesions on sBMD*. Tibial and femoral bone marrow lesions are positively associated with sBMD and this appears to be stronger in women compared with men.

Table 4.4. Multivariable analysis examining structural associations with medial sBMD*

	Partial correlation coefficients (r)	
	ROI 1	ROI 2
Tibial osteophytes (0–3)	**0.08***	**0.08***
Femoral osteophytes (0–3)	0.05	0.06
JSN (0–3)	**0.11****	0.03
Cartilage defects (0–8)	**0.16****	**0.17****
Cartilage volume (mL)	**0.12***	0.08
Tibial bone area (mm^2)	0.001	0.03
Tibial BMLs (0–3)	**0.17***	**0.19****
Femoral BMLs (0–3)	**0.16***	**0.17***
Spine BMD (g/cm^2)	**0.38****	**0.39****
Hip BMD (g/cm^2)	**0.36****	**0.39****

sBMD*: transformed subchondral bone mineral density; ROI: region of interest; JSN: joint space narrowing; BMLs: bone marrow lesions; BMD: bone mineral density.
Values represent medial measurements.
Adjusted for age, sex, and BMI.
Bold denotes a statistically significant result (*$P < 0.05$, **$P < 0.01$).

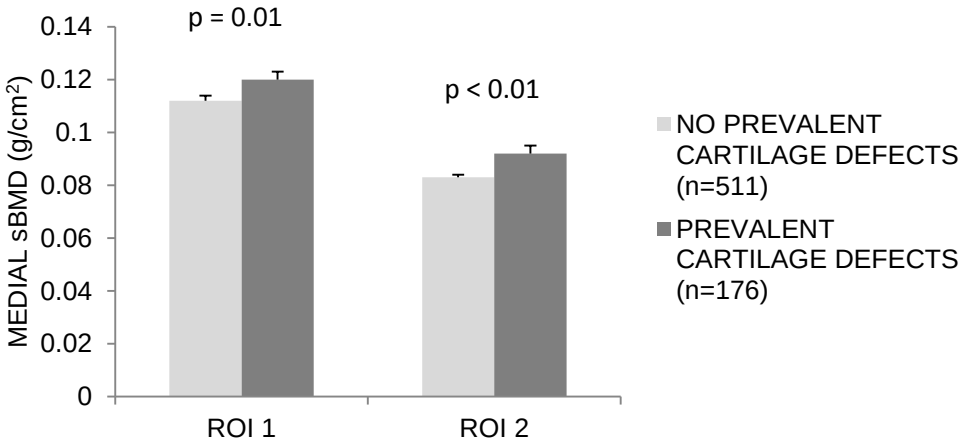
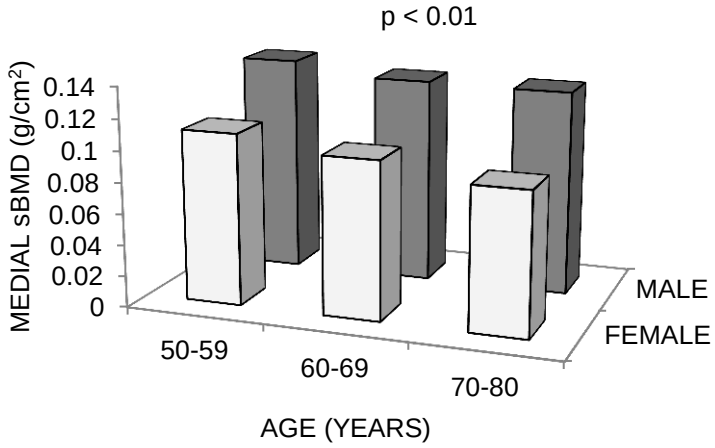
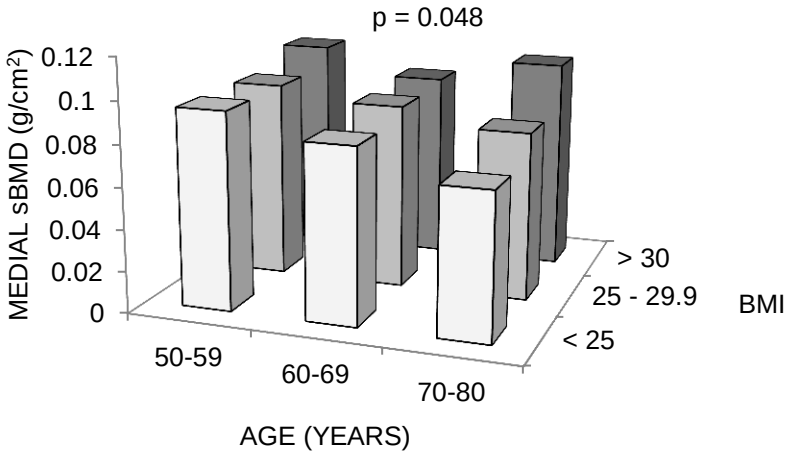


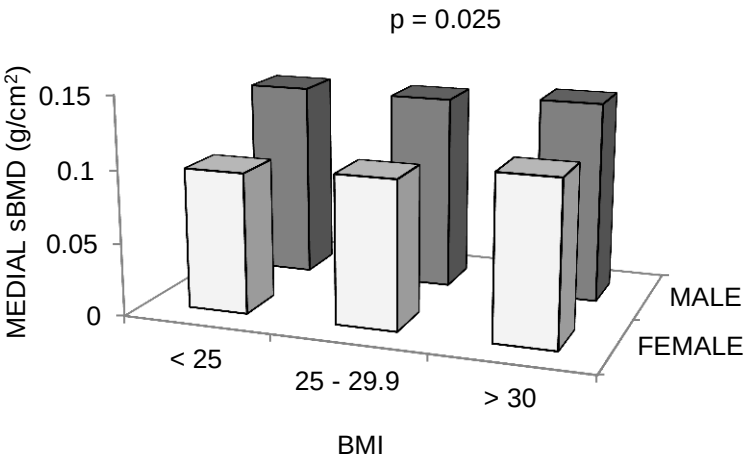
Figure 4.2. Mean difference in medial sBMD* between those subjects with and without prevalent cartilage defects using ROIs 1 and 2. Error bars represent standard error.



(A)

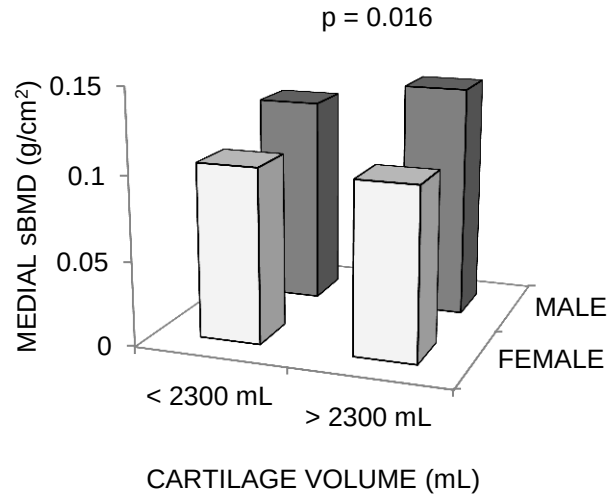


(B)

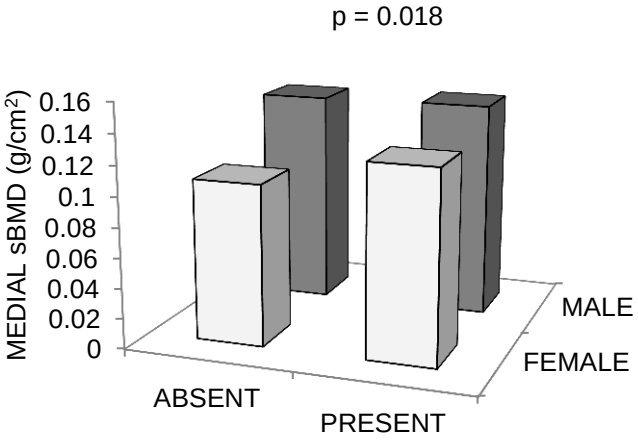


(C)

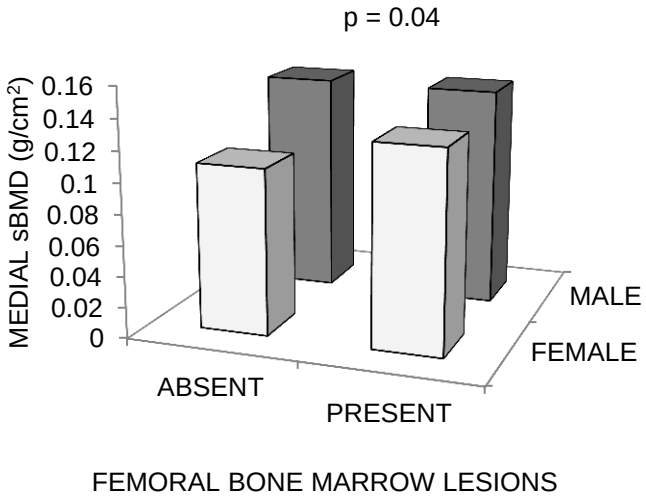
Figure 4.3. Interactions between age, sex, and BMI on medial sBMD*. Adjusted for age, sex, and BMI.



(A)



(B)



(C)

Figure 4.4. Interactions between sex and structural features on medial sBMD* using ROI 1. Adjusted for age, sex, and BMI.

4.3.5 Lateral sBMD and lateral structural measures

Similar results were observed for lateral sBMD* and lateral structural features. After adjustment for age, sex, and BMI, lateral sBMD* using ROI 1 and 2 was associated with cartilage defects (r = 0.18, $P < 0.01$; r = 0.13, $P < 0.01$, respectively), tibial (r = 0.13, $P < 0.01$; r = 0.13, $P < 0.01$, respectively) and femoral (r = 0.08, $P = 0.043$; r = 0.11, $P < 0.01$, respectively) osteophytes, and spine (r = 0.34, $P < 0.01$; r = 0.23, $P < 0.01$, respectively) and hip (r = 0.34, $P < 0.01$; r = 0.27, $P < 0.01$, respectively) BMD. Additionally, lateral sBMD* was positively associated with tibial bone area (r = 0.20, $P < 0.01$; r = 0.15, $P = 0.025$) using ROI 1 and 2. Tibial BMLs were associated with lateral sBMD* using ROI 1 (r = 0.15, $P = 0.023$).

4.4 Discussion

This cross-sectional study identified many factors associated with sBMD including age, sex, BMI, vitamin D, sun exposure, physical activity, and many knee structural features. Of these the most novel is cartilage defects which were shown to be independently associated with sBMD, indicating a possible causal relationship. The relatively modest correlation between sBMD and spine and hip BMD suggests that sBMD may have different determinants to BMD at other sites. Additionally, the region specific variations within the knee suggest that sBMD at different regions may have a varying response to different determinants.

Radin and Rose (98) hypothesised many years ago that subchondral bone plays an important role in OA pathogenesis. Cartilage defects are a major cause of cartilage loss (145, 146); thus, are an early marker of cartilage loss and eventually ROA. Recently, we demonstrated that subchondral bone expansion predicted cartilage defect development using a longitudinal design (209). This supported the hypothesis of Radin and Rose, but it is unknown whether this is due to increases in bone size, bone density, or both. In this study, medial and lateral sBMD were associated with a large number of structural features measured by x-ray and MRI, such as tibial osteophytes, JSN, cartilage defects, cartilage volume, and BMLs. Of these, the one that is most interesting is cartilage defects. This association persisted after adjustment for tibial bone size and tibial and femoral BMLs, suggesting cartilage defects are independently associated with sBMD. This could be causally related, indicating that denser subchondral bone may increase the risk of defect development. Given the cross-sectional nature of this study it remains possible that subchondral bone will increase density to cope with cartilage defects, although this seems less likely. Further longitudinal studies will shed light on causal pathways. The association between medial and lateral sBMD and osteophytes may be artefactual as has been reported at the spine (212). Spine BMD was shown to be associated with tibial osteophytes cross-sectionally; however, longitudinal designs have shown no difference in spine BMD between those participants whose osteophytes progressed versus those whose osteophytes did not progress (128). Osteophytes represent increased bone area and sBMD is an areal measure of bone density; therefore, it is expected that there would be an association between the two. ROI 1 included the intercondylar spine and tibial plateau; thus, most likely included osteophytes in the measurement. However, osteophytes were also associated with sBMD using ROI 2 (which excluded the tibial plateau and was unlikely to include osteophytes in the measurement). This suggests that this association is not just due

to the effect of bone area, but that increases in sBMD and osteophyte formation may be under the same physiologic control. The association with BMLs has been reported previously (137). The reason for the higher sBMD is unclear but may reflect inflammatory changes in the bone leading to increased density. There was an interaction between sex and BMLs on medial sBMD. It seems that the positive association that exists between BMLs and sBMD is more pronounced in women than men.

The positive association between medial sBMD and both JSN and cartilage volume (using ROI 1) is unexpected and somewhat contradictory, given that cartilage volume decreases as JSN increases. However, it has been hypothesised that cartilage swelling may be an early manifestation of OA and lead to cartilage volume loss. This is supported by studies which have shown increased cartilage volume at baseline predicts greater cartilage volume loss (213, 214). This is also consistent with an experimental observation in a guinea pig model which found tibial cartilage swelling (expressed as greater cartilage volume) occurs in early OA, followed by cartilage fragmentation and degeneration (215). Therefore, those subjects with increased medial sBMD may have swollen cartilage, which will eventually lead to cartilage loss as the disease progresses. There was an interaction between sex and cartilage volume on medial sBMD. The positive association between cartilage volume and sBMD appears to be stronger in men than women.

Knee OA more commonly involves the medial tibiofemoral compartment (216); therefore, in this study we chose to focus more on medial sBMD. We confirmed expected associations between medial sBMD and factors such as age, sex, BMI, vitamin D, sun exposure and physical activity. These factors have also been reported to be associated with hip and spine BMD (122, 217-221). In a small pilot study from our institution, medial sBMD was associated with sex and height; however, we were unable to find an association between medial sBMD and age, weight or BMI, possibly due to sample size considerations (134). This study now confirms that medial sBMD is also associated with age, weight, and BMI in a similar way to that reported for hip and spine BMD. However, after adjustment for age and sex, the association between medial sBMD and height did not persist. There were interactions present between age and sex, age and BMI, and sex and BMI on medial sBMD. Age is negatively associated with sBMD but this appears to be stronger in women than men. The interaction between age and BMI is interesting, suggesting a steeper increase in sBMD as BMI increases in older adults, compared to the younger age categories. Similarly, there seems to be a steeper increase in sBMD as BMI increases in women, compared to men.

Steps/day was also associated with medial sBMD perhaps indicating a response to mechanical stressors. In a concurrent study in this population, we also found that hip BMD was positively associated with steps/day (218). This indicates that those participants who do more steps/day have a higher BMD, and this holds true for medial tibial sBMD as well. However, this may not be beneficial for OA given that medial sBMD was positively associated with cartilage defects.

The strong correlation between spine and hip BMD has been well documented. For example, in this study the correlation coefficient for the association between spine and hip BMD was 0.7. Both medial and lateral sBMD were moderately associated with spine and hip BMD; however, the correlation between them was not as strong as expected. The correlation coefficients for the association between sBMD and spine and hip BMD ranged from 0.3–0.5 at medial and lateral ROI 1 and 2. This somewhat modest correlation, along with the variations in associations between ROI 1 and ROI 2, suggest that subchondral bone is under substantially independent regulation to other sites.

Most OA studies have focused on symptomatic and/or diseased populations; therefore, this community-based sample is unique. The findings are generalisable to community-based older adults, and not just those with OA. OA cohorts are commonly selected based on the presence of radiographic OA; therefore excluding those with less advanced disease. Our cohort has the ability to study many disease stages as it includes those with no disease, those with early stage disease, and those with definite OA.

There are a number of potential limitations to the current study. First, immediate reproducibility for this method of measurement was excellent; however, interval reproducibility (measures repeated after at least 14 days) could be improved. Because measurement error may have implications in follow-up studies, it is suggested that methods be developed to minimise drift in measurements, such as a ROI atlas. Second, sBMD measured by DXA assesses ‘areal BMD’ and thus only evaluates bone in two dimensions. Other techniques will be required to assess ‘true volumetric density’. Lastly, the cross-sectional nature of this study precludes any inference about cause and effect relationships. Longitudinal studies are required to address causality.

In conclusion, the main hypothesis which has been generated from this study is denser subchondral bone may lead to cartilage defect development. Future research should focus on whether elevated sBMD predicts development or progression of cartilage defects. We also hypothesise that the presence of cartilage swelling is an early manifestation of OA, and although sBMD was positively associated with cartilage volume, further research should examine whether elevated sBMD is a risk factor for cartilage loss. Lastly, we

hypothesise that sBMD is under independent regulation to bone at other sites. sBMD was associated with many features of OA, possibly making it an ideal target for therapeutic intervention in OA.

Chapter 5 - Subchondral bone and cartilage damage: a prospective study in older adults

5.1 Introduction

Osteoarthritis (OA) is a complex disease characterised by involvement of multiple tissues in the synovial joint. It has long been hypothesised that subchondral bone is important in the pathogenesis of OA, but it remains controversial whether subchondral bone abnormalities precede cartilage damage in the early stages of the disease. In an earlier study we demonstrated that an elevated tibial bone area predicted cartilage defect development over time in younger, healthy individuals (139). Additionally Davies-Tuck et al (144) confirmed that an elevated tibial bone area is a risk factor for defect progression in those with well-established symptomatic knee OA. This suggests that increased knee bone size is causally related to knee cartilage defects. However, it is unknown whether this is due to increases in bone size, bone density, or both. Cartilage defects are also described as a major contributor to cartilage volume loss (145, 146); thus, it is reasonable to hypothesise that an elevated tibial bone area will also predict cartilage volume loss; although studies have failed to demonstrate this to date (147-149).

Subchondral bone mineral density (sBMD) is thought to play a role in OA pathology. There are conflicting data as to whether the density of the subchondral bone is elevated in OA. Some studies have demonstrated lower than normal bone mineral density (BMD) in the subchondral regions of patients with knee OA (135, 222). Conversely, studies have found that an elevated sBMD is associated with radiographic osteoarthritis (ROA); including osteophyte formation, joint space narrowing (JSN), and subchondral sclerosis (132, 136). Longitudinally, it has been shown that those patients with the highest tibial sBMD at baseline experienced the most dramatic JSN after 1 year (133). This lends support to the hypothesis that an elevated sBMD predicts cartilage volume loss. Chapter 4 suggested that sBMD is independently associated with cartilage defects after taking bone area into account but this needs to be confirmed longitudinally.

Therefore, the aim of the current study is to describe the association between tibial bone area and tibial sBMD with cartilage defect development and cartilage volume loss over approximately 2.7 years in a population-based random sample of older men and women.

5.2 Materials and Methods

5.2.1 Knee cartilage volume

Medial and lateral tibial cartilage volume was determined at baseline and follow-up as described in section 3.5.4. Absolute change in cartilage volume was calculated as: follow-up cartilage volume - baseline cartilage volume. Rate of change in cartilage volume was calculated as: percentage change per annum

$$\text{pa} = \left[\frac{\text{absolute change / baseline cartilage volume}}{\text{time between 2 scans, in years}} \right] \times 100.$$

5.2.2 Knee cartilage defects

Medial and lateral tibial cartilage defects were assessed at baseline and follow-up as described in section 3.5.3. The images were read unpaired; therefore, they were not blinded to order. However, the reader was unaware of the baseline result at the time of second reading. An increase in cartilage defect score from baseline to follow-up was defined as an increase of one or more on the 0–4 scale. Those whose scores remained the same or decreased by one or more were defined as stable or decreasing.

5.2.3 Tibial subchondral bone mineral density

Baseline medial and lateral sBMD of the tibia was measured using three regions of interest (ROI) as described in section 3.4 and displayed in Figure 3.2.

5.2.4 Statistical analysis

For graphical purposes, sBMD and bone area were split into quartiles and correlation analysis was used to compare the average cartilage lost per annum between each quartile, for univariate analysis.

Logistic regression analysis was used to examine the multivariable associations between sBMD and bone area and increases in cartilage defects (increase versus no increase) after adjustment for age, sex, body mass index (BMI), baseline defect score, and sBMD if bone area and bone area if sBMD. Standard diagnostic checks of model adequacy and unusual observations were performed. Hosmer-Lemeshow tests were performed to assess goodness-of-fit. Generalised estimating equations (GEE) were used to examine the association between the baseline bone measures and both percentage change per annum

and absolute volume change, after adjustment for age, sex, BMI, baseline defect score, baseline cartilage volume, and sBMD if bone area and bone area if sBMD.

5.3 Results

5.3.1 Subjects

The current study consists of a sample of 341 participants (mean age 63 years, range 52–79 years, 48% female) who had DXA measures at baseline and magnetic resonance imaging (MRI) measures at baseline and follow-up. The range of follow-up times was 1.9–3.7 year (mean approximately 2.7 years). The majority of participants (90%) were followed up between 2.2–3.2 years. There were no significant differences in demographics or baseline sBMD, cartilage defects, or cartilage volume between the rest of the cohort (n=758) and the subjects included in the current study (n=341); although, there was a small difference in baseline bone area between the subjects in the current study [mean bone area 2,130 mm^2 (standard deviation (SD) 311)] compared with the rest of the cohort [mean bone area 2067 mm^2 (SD 304); $P < 0.01$ for difference]. The characteristics of the study population at baseline are presented in Table 5.1. There were no significant associations between sBMD and cartilage damage (defect development or volume loss) using region of interest (ROI) 1 or 2 (data not shown); therefore, all the sBMD results reported are using ROI 3.

At baseline, sBMD was not associated with bone area at the medial tibial site (r = 0.02, $P = 0.67$) and was modestly but significantly associated at the lateral tibial site (r = 0.14, $P = 0.01$), after adjustment for age, sex, and BMI.

At follow-up, at the medial tibial site, 2 subjects had a decrease in defect score, 286 remained stable, and the score increased in 53 subjects. At the lateral tibial site, 5 participants had a decrease in defect score, 278 remained stable, and 58 had scores that increased.

Table 5.1. Characteristics of the participants at baseline*

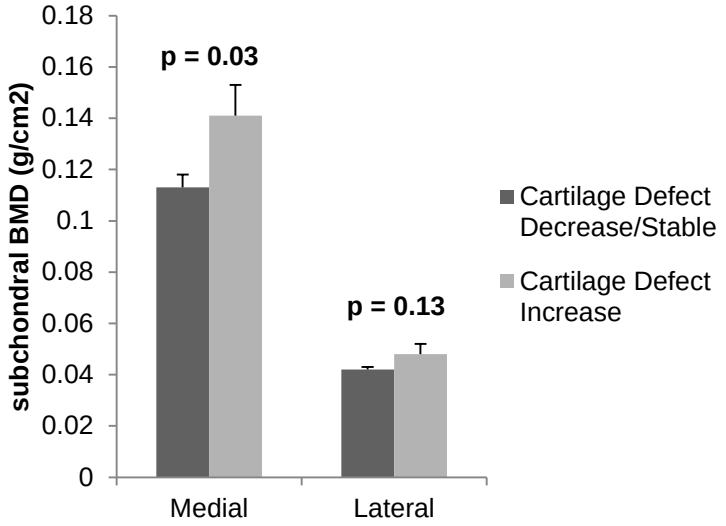
	Medial site				Lateral site			
	Defect decrease/ stable (n = 288)	Defect increase (n = 53)	Volume loss less than the mean (n = 163)	Volume loss equal to or greater than the mean (n = 169)	Defect decrease/ stable (n = 283)	Defect increase (n = 58)	Volume loss less than the mean (n = 168)	Volume loss equal to or greater than the mean (n = 164)
Age (year)	63.0 (7.2)	64.3 (7.5)	62.1 (6.8)	64.1 (7.4)†	63.3 (7.3)	63.0 (6.7)	62.5 (7.0)	63.9 (7.3)
Male sex (%)	52	53	46	57‡	52	50	55	48
BMI (kg/m^2)	27.4 (4.4)	28.6 (4.7)	27.1 (4.3)	27.9 (4.3)	27.4 (4.3)	28.1 (4.9)	27.4 (4.6)	27.7 (4.1)
sBMD (g/cm^2)ϕ	0.113 (0.082)	0.141 (0.089)‡	0.112 (0.065)	0.121 (0.094)	0.042 (0.024)	0.048 (0.030)	0.043 (0.023)	0.043 (0.025)
Bone area (mm^2)	2,118 (307)	2,199 (329)	2,059 (276)	2,201 (326)†	1,215 (207)	1,271 (199)	1,225 (205)	1,222 (206)
Cartilage defects present (%)Δ	8	23†	6	15†	15	14	14	14
Cartilage volume (mL)	2,358 (603)	2,261 (549)	2,119 (520)	2,551 (581)†	2,825 (718)	2,628 (626)	2,748 (721)	2,842 (693)

*Except where indicated otherwise, values are the mean (standard deviation). The mean volume change was -2.5% per annum at the medial site and -2.0% per annum at the lateral site. *P* values were determined by t-test or chi-square test (where appropriate). BMI: body mass index; sBMD: subchondral bone mineral density.
†$P < 0.01$ versus defect decrease/stable or volume loss less than the mean.
‡$P < 0.05$ versus defect decrease/stable or volume loss less than the mean.
ϕUsing region of interest 3.
ΔDefined as grade 2 or higher.

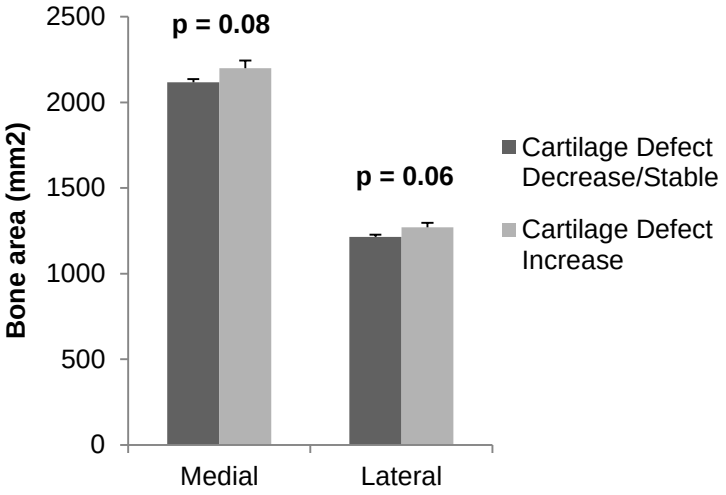
5.3.2 Tibial cartilage defect increase

Figure 5.1 shows the univariate relationship between tibial sBMD and tibial bone area and cartilage defect increases. Medial tibial sBMD was higher in those participants whose cartilage defects increased versus those whose decreased or remained stable. There was no significant association between lateral tibial sBMD and cartilage defect changes. There was a trend towards higher medial and lateral tibial bone area in those participants whose cartilage defects increased versus those whose decreased or remained stable.

Table 5.2 shows the multivariable relationship between tibial sBMD and tibial bone area and cartilage defect increases. Tibial bone area predicted defect increases at both the medial and lateral sites. Tibial sBMD predicted defect increases at the medial site only. These results were similar in less adjusted analysis, where sBMD was not adjusted for bone area and bone area was not adjusted for sBMD (data not shown). There was no interaction between sBMD and bone area on cartilage defect progression.



(A)



(B)

Figure 5.1. Mean baseline (A) subchondral bone mineral density (sBMD) and (B) bone area in subjects whose cartilage defects decreased or remained stable and subjects whose cartilage defects increased. Error bars represent standard error.

Table 5.2. Associations of baseline bone measures with increases in cartilage defects during 2.7 years*

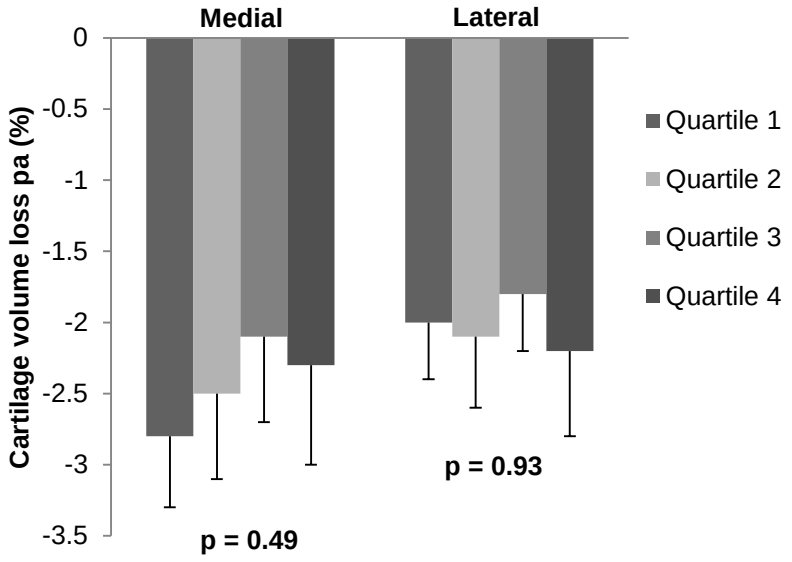
	Multivariable OR (95% CI)†	*P*
Medial tibial defect increase		
Medial tibial sBMD, per SD	**1.6 (1.2, 2.1)**	**<0.01**
Medial tibial bone area, per SD	**1.6 (1.0, 2.6)**	**0.04**
Lateral tibial defect increase		
Lateral tibial sBMD, per SD	1.2 (0.9, 1.6)	0.19
Lateral tibial bone area, per SD	**2.4 (1.4, 4.0)**	**<0.01**

*Stable/decreased defects are coded as '0' and an increase is coded as '1'.
OR: Odds ratio; 95% CI: 95% confidence interval; sBMD: subchondral bone mineral density; SD: standard deviation.
Bold denotes a significant result.
†Adjusted for age, sex, body mass index, baseline cartilage defects, and sBMD if analysing bone area and bone area if analysing sBMD.

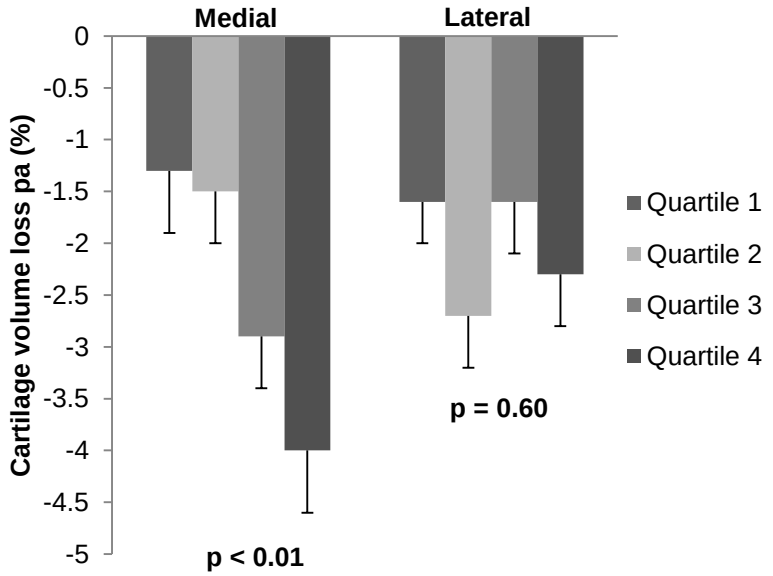
5.3.3 Tibial cartilage volume loss

Figure 5.2 shows the univariate relationship between quartiles of tibial sBMD and tibial bone area and percentage cartilage volume loss per annum. There was no difference in the quartiles of tibial sBMD and cartilage loss at the medial or lateral sites. There was a dose-response relationship between the quartiles of medial tibial bone area and medial cartilage loss. Lateral tibial bone area was not associated with lateral cartilage loss.

Table 5.3 shows the multivariable relationship between tibial sBMD and tibial bone area and absolute cartilage volume loss. Tibial bone area predicted cartilage loss at the medial site only. Tibial sBMD did not predict medial or lateral cartilage loss. These results were similar in less adjusted analysis, where sBMD was not adjusted for bone area and bone area was not adjusted for sBMD (data not shown). The same results were found using percentage cartilage volume loss per annum (data not shown). There was no interaction between sBMD and bone area on cartilage volume loss.



(A)



(B)

Figure 5.2. Mean cartilage loss per annum for each quartile of baseline (A) subchondral bone mineral density (sBMD) and (B) bone area. Error bars represent standard error. *P* values are for tests of trend.

Table 5.3. Associations of baseline bone measures with absolute changes in cartilage volume during 2.7 years*

	Multivariable β (95% CI)†	*P*
Medial tibial cartilage volume change		
Medial tibial sBMD (g/cm^2)	6.7 (- 7.7, 21.1)	0.36
Medial tibial bone area (mm^2)	**- 34.9 (- 49.8, - 20.1)**	**<0.01**
Lateral tibial cartilage volume change		
Lateral tibial sBMD (g/cm^2)	- 6.6 (- 20.6, 7.5)	0.36
Lateral tibial bone area (mm^2)	- 6.8 (- 20.4, 6.8)	0.33

*The beta coefficients were per 1 standard deviation increase in volume.
β: Beta coefficient; 95% CI: 95% confidence interval; sBMD: subchondral bone mineral density.
Bold denotes a significant result.
†Adjusted for age, sex, body mass index, baseline cartilage defects, baseline cartilage volume and sBMD if analysing bone area and bone area if analysing sBMD.

5.4 Discussion

This longitudinal study documents associations between cartilage defect development and cartilage loss and baseline bone area and sBMD. It demonstrates that bone area predicted medial and lateral cartilage defect increases and cartilage loss at the medial site, while sBMD predicted cartilage defect increases at the medial site but did not predict cartilage volume loss. These associations were independent of each other indicating there are multiple mechanisms by which subchondral bone may lead to cartilage damage.

Radin and Rose (98) hypothesised many years ago that subchondral bone plays an important role in OA pathogenesis. Recently, our group has shown in two separate studies that subchondral bone expansion predicted cartilage defect development using a longitudinal design (139, 144). However, it is unknown whether this is due to increases in bone size or likely correlates such as bone density, or both. The current study supports our previous findings, demonstrating that an elevated bone area predicted cartilage defect development and worsening. This association was seen after adjustment for sBMD and indeed there was a correlation between sBMD and bone area at baseline in the lateral compartment. This indicates that this relationship is independent of density. In addition, we observed that elevated sBMD predicted cartilage defect worsening at the medial site, independent of bone area. In our previous work, we demonstrated that both medial and lateral sBMD were independently associated with cartilage defects cross-sectionally (Chapter 4), supporting the hypothesis that denser subchondral bone may lead to defect development. In the current study, lateral sBMD was higher in those participants whose cartilage defects increased, but this was not statistically significant.

The association seen between sBMD and medial cartilage defect development was seen with ROI 3 only. ROI 1 included the intercondylar spine and ROI 2 did not include the subchondral cortical plate. This suggests that the area directly adjacent to the cartilage is more likely to be implicated in cartilage damage. ROI 1 and 2 both captured deeper areas of subchondral bone which were not included in ROI 3, suggesting that the bone in closer proximity to the joint surface may play a greater role in cartilage damage.

To the best of our knowledge, this is the first study to demonstrate that higher tibial bone area predicts cartilage volume loss. The effect was seen after adjustment for baseline defect score and cartilage volume, both of which have been shown to be predictors of cartilage loss (145, 146, 213). In contrast, elevated tibial bone area is associated with higher cartilage volume cross-sectionally (96), and this was also seen in the baseline data of this study (data not shown). The difference between cross-sectional and longitudinal

results could be explained by the hypothesis that the presence of cartilage swelling is an early manifestation of OA. This hypothesis is supported by studies which have shown increased cartilage volume at baseline predicts greater cartilage volume loss (213, 214). This is also consistent with an experimental observation in a guinea pig model which found tibial cartilage swelling (expressed as greater cartilage volume) occurs in early OA, followed by cartilage fragmentation and degeneration (215). Tibial bone expansion may be a primary event in OA and may induce increases in cartilage volume, such as those we observed at baseline, possibly due to cartilage swelling, which then leads to greater cartilage volume loss.

Although denser subchondral bone predicted medial defect development, sBMD did not predict cartilage volume loss in this study. This is unexpected given that previously it has been demonstrated that those patients with the highest tibial sBMD at baseline experienced the most dramatic JSN after 1 year (133). However, this could be explained by the hypothesis of cartilage swelling in early OA (139). We have previously shown that baseline cartilage volume was positively associated with increases in medial tibiofemoral defects (139) and increased cartilage volume at baseline predicted greater cartilage volume loss (213). Additionally, a cross-sectional study in this cohort found that baseline sBMD was positively associated with baseline cartilage volume (Chapter 4). In the current study, of those participants who increased in medial tibial cartilage defects, 66% of them went from a 1 to a 2 on the 0–4 scale. It could be possible that such early defects lead to cartilage swelling, and will eventually lead to cartilage loss as the disease progresses. Another possibility could be the transient nature of cartilage defects. While in this (Doré: unpublished observations) and other cohorts, cartilage defects predict cartilage loss (145, 146), cartilage defects can also resolve (139). Therefore, longer time frames may be required to see cartilage defects attributable to increased sBMD convert to cartilage loss.

This study has a few potential limitations. First, sBMD measured by DXA assesses ‘areal BMD’ and thus only evaluates bone in two-dimensions. Therefore it may be influenced by differences in bone size. However, we were able to adjust for tibial bone area, which assesses the third dimension of bone size. Additionally, the measurement of bone area and sBMD could be influenced by the presence of osteophytes. However, after further adjustment for osteophytes the results were unchanged. Second, the response rate for participation in the study was 57% at baseline. This response rate does leave the possibly open for selection bias; however, this is unlikely to bias the associations we report due to the method of analysis. The study also tended to have high rates of retention (80%) to offset this. For the current study, 759 were not included due to unread DXA scan results,

and the MRI scanner was no longer available for use in the study. There were no significant differences between those studied and the rest of the cohort in regards to demographics, baseline sBMD, cartilage defects, or cartilage volume. However, those studied had a significantly higher baseline tibial bone area. Third, we used tibial cartilage rather than femoral cartilage as the measure of joint cartilage at the tibiofemoral joint because we did not have measures of femoral sBMD and bone area. It is possible that associations with femoral cartilage damage are different to those with tibial cartilage damage. Fourth, we did not study bone attrition; however, we did have data on bone marrow lesions and found that our results remained unchanged after adjustment for bone marrow lesions (data not shown). Lastly, there has been subsequent communication about this study regarding the type of MRI sequences used to assess cartilage defects. Appendix 4 contains a letter to the editor by Hayashi et al and our reply is included.

In conclusion, bone area predicted medial and lateral cartilage defect development and medial cartilage volume loss, while sBMD predicted medial defect development but not cartilage loss. These associations were independent of each other, indicating multiple mechanisms by which subchondral bone may lead to cartilage damage and loss.

Chapter 6 - Bone marrow lesions predict site-specific cartilage defect development and volume loss: a prospective study in older adults

6.1 Introduction

Bone marrow lesions (BMLs), detected by magnetic resonance imaging (MRI), have been recognised as an important feature in knee osteoarthritis (OA) (150, 151). A number of studies have linked BMLs with knee pain (81, 150, 174, 175) although other studies have failed to demonstrate such a relationship (176-178). Baseline BMLs and increases in BML size have been shown to predict cartilage defect progression (158-161) and cartilage loss (158, 159, 162-167). However, most of these studies have used a compartment-level approach by combining tibial and femoral sites (158, 159, 162, 163, 165) and/or medial and lateral tibiofemoral compartments (158, 159). The relationship between BMLs and changes in site-specific cartilage has only recently been examined (164, 166, 167). Kothari et al (164) found that the presence of BMLs at baseline was associated with cartilage loss in the same subregion at 2 years. In another study, Roemer et al (166) examined BML changes with changes in cartilage over time. They reported that the absence of BMLs at baseline and follow-up was associated with a decreased risk of adjacent cartilage loss, while new or progressive BMLs displayed a high risk of adjacent cartilage loss (166). Cartilage scores in both of these studies were assessed using the Whole-Organ Magnetic Resonance Imaging Score (WORMS) method, which semi-quantitatively scores cartilage integrity by using one scale for both cartilage defects and cartilage loss. Alternatively, Raynauld et al (167) examined the subregional relationship between BMLs with a quantitative measure of cartilage volume loss and found that an increase in bone edema was associated with cartilage volume loss in same subregions of the medial but not lateral compartment. Therefore there is increasing evidence to demonstrate that BMLs predict site-specific cartilage changes; however, it remains unclear whether BMLs at one site predict cartilage changes in another.

There is an ongoing debate about the role BMLs play in the development of cartilage damage and loss. It remains unclear whether BMLs precede, accompany, or follow cartilage damage and volume loss in OA (164). Many studies have shown that baseline BMLs predict subsequent cartilage damage and/or loss (158-160, 162-165); however, to the best of our knowledge, there have been no studies examining whether baseline cartilage defects predict BML progression.

Therefore, the aims of this population-based longitudinal study were to examine: 1) the relationship between baseline BMLs and site-specific changes in cartilage (defects and/or volume changes); 2) whether baseline BMLs at one site predict cartilage changes

(defects and/or volume changes) in another; and 3) whether baseline cartilage defects predict site-specific BML progression.

6.2 Materials and Methods

6.2.1 Knee cartilage volume

Tibial cartilage volume was assessed as described in section 3.5.4. Knee femoral cartilage volume was assessed by means of image processing on an independent workstation using Cartiscope™ (ArthroVision Inc., Montreal, Quebec, Canada), as previously described (223-225). The segmentation of the cartilage-synovial interfaces was carried out with a semi-automatic method under reader supervision and with corrections when needed. Cartilage volume was evaluated directly from a standardised view of three-dimensional (3D) cartilage geometry as the sum of elementary volumes. The coefficient of variation (CV) was approximately 2% (223). The cartilage volume assessment was done for the medial and lateral condyles delineated by the Blumensaat's line (224). Absolute change in cartilage volume was calculated as: follow-up cartilage volume - baseline cartilage volume. Rate of change in cartilage volume was calculated as: percentage change per annum

$$\text{pa} = \left[\frac{\text{absolute change / baseline cartilage volume}}{\text{time between 2 scans, in years}} \right] \times 100.$$

6.2.2 Knee cartilage defects

Cartilage defects were assessed as described in section 3.5.3. Cartilage defect progression was defined as an increase of one or more on the 0–4 scale. Those whose scores remained the same or decreased by one or more were defined as stable or decreasing.

6.2.3 Bone marrow lesions

BMLs were assessed using the ordinal scoring system as described in section 3.5.2. BML progression was defined as an increase of one or more on the 0–3 scale. Those whose scores remained the same or decreased by one or more were defined as stable or decreasing.

In an extended observation, BMLs were also scored using a modified version of WORMS by a separate research group, in order to compare the two scoring systems. Briefly, BMLs were assessed on T1-weighted MR images and the joint was divided into its anatomical regions (medial and lateral condyle, medial and lateral tibial plateau, and

patella), which were further subdivided into anterior, central, and posterior for the femur, and medial and lateral for the patella and the tibial plateaus. Subchondral bone marrow abnormalities were then assessed comparing the surface of the lesion with the surface of the subregion in the corresponding image. If the lesion was depicted in multiple slides, the one with the largest extent was chosen. When the lesion is oriented along the latero-medial direction, a reconstructed axial image is used for the evaluation. A scale from 0–3 was used, where 0 = absence, 1 = <25%, 2 = 25% to 50%, and 3 = >50% of this ratio. The central and posterior femoral subregions and the tibial plateau formed the medial and lateral compartments. The medial and lateral anterior femoral subregions and the two patellar subregions formed the femoropatellar compartment. The interreader reliability of this BML scoring system has previously been shown to be excellent (167).

6.2.4 Meniscal damage evaluation

Meniscal damage evaluation at baseline was performed by a trained observer as previously described (225). In brief, the proportion of the menisci affected by the tear or extrusion was separately scored on the medial and lateral edges of the tibiofemoral joint space using a semi-quantitative scale. For tears the following scale applied: 0 = no damage, 1 = one of three areas involved (anterior, middle, posterior horns), 2 = two of three involved, 3 = all three areas involved. The extent of meniscal extrusion, not including the osteophytes, was evaluated for the anterior, middle, and posterior horns of the menisci in which 0 = no extrusion, 1 = partial extrusion and 2 = complete extrusion with no contact with the joint space (severe).

Cartilage volume measurements, cartilage defects, BMLs, and meniscal damage scoring were all done independently to one another.

6.2.5 Statistical analysis

Site-specific associations were defined as the associations within the same site (example, the association between medial tibial BMLs and medial tibial defect increases). Compartment-specific associations were defined as the associations within the same compartment (example, the association between medial tibial BMLs and medial femoral defect increases).

Due to a lack of variation in baseline cartilage defect score in this cohort, cartilage defects were dichotomised for some analyses. Defect scores of 0–1 were coded 0 and of 2–4 were coded 1.

Logistic regression modeling was used to examine the site and compartment-specific associations between baseline BMLs with increases in cartilage defects (increase versus no increase) and baseline defects with increases in BMLs (increase versus no increase), after adjustment for age, sex, body mass index (BMI), and defects if BMLs and BMLs if defects. As there is increasing evidence to suggest that meniscal damage plays an important role in disease progression, models were further adjusted for meniscal damage. Meniscal damage has been shown to predict cartilage loss (163, 225) and BML development (226, 227). Therefore it is believed that meniscal pathology, cartilage damage, and BMLs are all related, although the time sequence of these pathological events is still unclear. By further adjusting for meniscal damage we were able to assess whether the associations between BMLs and cartilage defects were independent of meniscal pathology. Due to the uncertainty of the chronological order of these features, we have chosen to display both the unadjusted and adjusted results. Standard diagnostic checks of model adequacy and unusual observations were performed. Hosmer-Lemeshow tests were performed to assess goodness-of-fit.

Generalised estimating equations (GEE) were used to examine the site and compartment-specific associations between baseline BMLs and cartilage defects with change in absolute cartilage volume after adjustment for age, sex, BMI, baseline site-specific cartilage volume, and defects if BMLs and BMLs if defects. Models were then further adjusted for meniscal damage to assess the independent effects of BMLs and cartilage defects on cartilage volume loss. The interaction between baseline BMLs and baseline defects on cartilage volume loss was also examined.

6.3 Results

6.3.1 Subjects

The current study consists of a sample of 405 participants (mean age 63 years, range 52–79 years, 48% female) who had MRI measures at baseline and follow-up. The range of follow-up was 2.0–4.7 years (mean ~ 2.7 years). The majority of participants (90%) were followed up between 2.2–3.2 years. MRI scans were discontinued after this sample due to decommissioning of the MRI scanner. There were no significant baseline differences in demographics, cartilage defects, BMLs, and cartilage volume between the rest of the cohort (n=694) and the subjects included in the current study (n=405).

The characteristics of the study sample by presence or absence of baseline BMLs at any site are presented in Table 6.1. At all four sites, in unadjusted analysis, subjects who had a BML at baseline had a higher prevalence of baseline cartilage defects, lost more cartilage volume from baseline to follow-up, and a higher proportion of them increased in cartilage defects from baseline to follow-up, compared with those subjects who did not have a BML at baseline. There was limited variation in baseline cartilage defect scores. No participants scored zero at the medial or lateral tibial sites. The majority of participants scored one and smaller numbers of participants scored greater than two at all four sites.

Table 6.1. Characteristics of participants according to presence or absence of BMLs at baseline at each site*

	Medial tibial		Medial femoral		Lateral tibial		Lateral femoral	
	BML absent (n = 352)	BML present (n = 53)	BML absent (n = 358)	BML present (n = 47)	BML absent (n = 379)	BML present (n = 26)	BML absent (n = 356)	BML present (n = 49)
Age (year)	63.2 (7.2)	63.5 (7.2)	63.3 (7.3)	62.7 (6.3)	63.1 (7.3)	65.0 (6.4)	63.3 (7.2)	63.0 (7.6)
Male sex (%)	49	53	49	57	50	46	**47**	**65†**
Body mass index (kg/m^2)	27.6 (4.4)	28.0 (4.8)	27.5 (4.5)	28.5 (4.0)	27.7 (4.5)	26.6 (2.7)	27.7 (4.5)	27.2 (4.1)
Cartilage defects present baseline (%)ϕ	**7**	**28‡**	**15**	**43‡**	**15**	**38‡**	**5**	**31‡**
Cartilage defect increase (%)	**13**	**25†**	**23**	**44‡**	**14**	**52‡**	**17**	**41‡**
Cartilage volume baseline (mL)	2332 (580)	2352 (563)	3949 (1135)	4024 (1089)	2763 (681)	2687 (807)	4327 (1194)	4351 (876)
Cartilage volume loss per annum (%)	**-2.3 (5.3)**	**-4.4 (5.1)†**	**-1.1 (2.1)**	**-2.2 (2.9)‡**	**-1.8 (4.0)**	**-4.7 (6.2) ‡**	**-0.8 (2.0)**	**-1.7 (1.9)†**
BML increase (%)	11	17	**5**	**15†**	11	12	**12**	**27‡**

*Mean (standard deviation) except for percentages. *P* values determined by t-test or chi-square test (where appropriate).

BMLs: bone marrow lesions.

Bold denotes a statistically significant result.

†$P < 0.05$.

‡$P < 0.01$.

ϕDefined as grade 2 or higher.

6.3.2 BMLs and cartilage defects

Site-specific associations

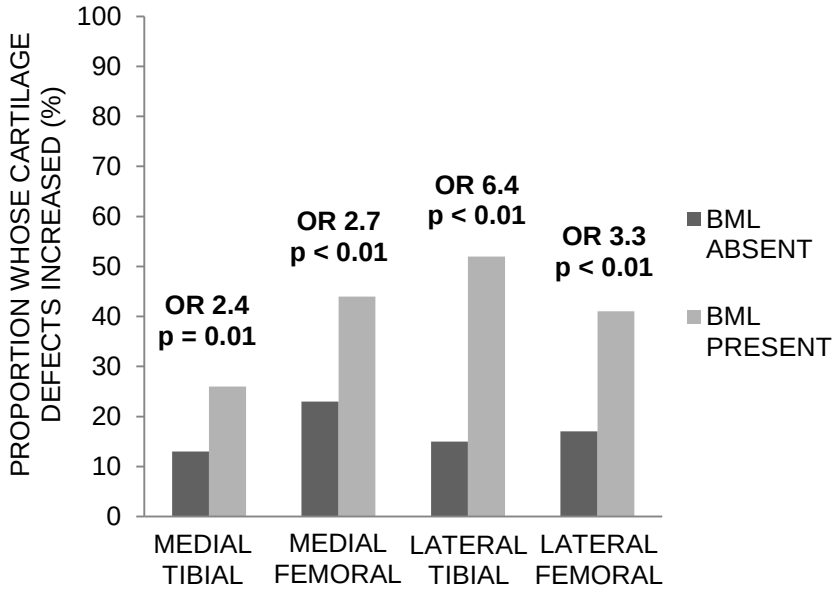
Figure 6.1 describes the site-specific univariate relationship between (A) baseline BMLs and cartilage defect increases and (B) baseline cartilage defects and BML increases. There were a higher proportion of participants whose cartilage defects increased in those with a BML at baseline versus those without a BML at baseline (A). There were also a higher proportion of participants whose BMLs increased in those with baseline defect grades 2–4 versus those with defect grades 0–1 (B).

Table 6.2 describes the multivariable relationship between baseline BML severity and cartilage defect increases and baseline cartilage defect severity and BML increases. BMLs predicted site-specific cartilage defect increases in a dose-response fashion at each site, even after further adjustment for meniscal damage. For example, at the medial tibial site, the odds of a cartilage defect increasing opposed to not increasing was 1.8 times more per grade increase in baseline BML score. Cartilage defect severity predicted site-specific increases in BMLs in a dose-response manner also at each site; however, after further adjustment for meniscal damage this only persisted at the medial tibial and lateral femoral sites.

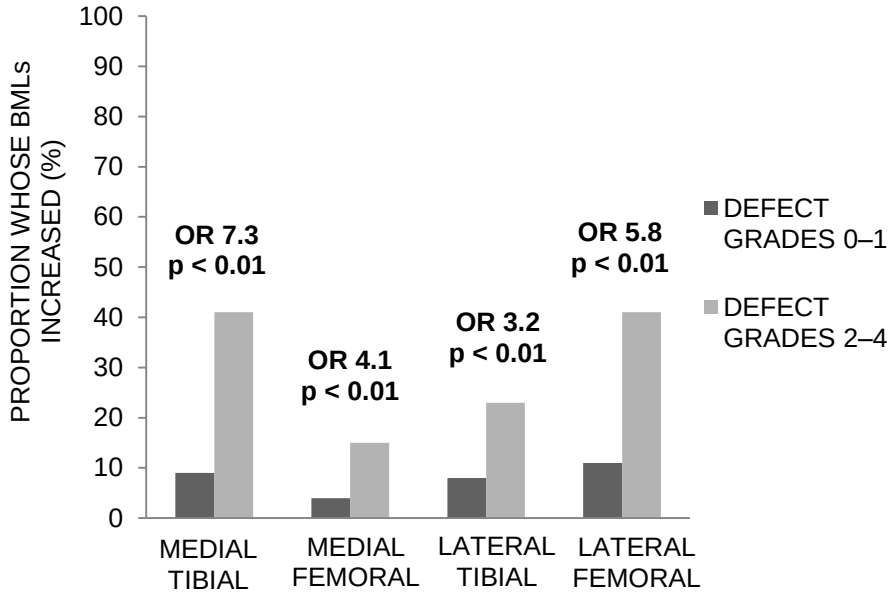
Compartment-specific Associations

Medial femoral BMLs predicted medial tibial cartilage defect increases (odds ratio (OR) 1.7, 95% CI 1.1–2.7), and this persisted after further adjustment for medial tibial BMLs and meniscal damage (OR 1.9, 95% CI 1.2–3.0). BMLs did not significantly predict compartment-specific defect increases at any other site.

Lateral tibial defects predicted lateral femoral BML increases (OR 2.3, 95% CI 1.5–3.7), and this persisted after further adjustment for lateral femoral defects and meniscal damage (OR 2.3, 95% CI 1.1–4.7). Defects did not significantly predict compartment-specific BML increases at any other site.



(A)



(B)

Figure 6.1. Baseline BMLs with cartilage defect increases and baseline cartilage defects with BML increases by site. (A) Proportion of participants whose cartilage defects increased in those with no baseline BML versus those with a baseline BML. (B) Proportion of participants whose BMLs increased in those with baseline cartilage defect grades 0–1 versus those with baseline cartilage defect grades 2–4.

Table 6.2. Association between baseline BMLs (0–3) and site-specific increases in cartilage defects at the same site and baseline cartilage defects (0–4) and site-specific increases in BMLs at the same site

	Multivariable OR (95% CI)†	Multivariable OR (95% CI)‡
BMLs predicting defect increases		
Medial tibial BMLs	**1.8 (1.2, 2.7)****	**1.8 (1.1, 2.9)***
Medial femoral BMLs	**2.3 (1.5, 3.5)****	**2.2 (1.4, 3.5)****
Lateral tibial BMLs	**2.8 (1.8, 4.5)****	**3.2 (1.9, 5.4)****
Lateral femoral BMLs	**3.3 (2.1, 5.0)****	**3.0 (1.9, 4.8)****
Defects predicting BML increases		
Medial tibial defects	**3.7 (2.1, 6.5)****	**3.3 (1.6, 6.8)****
Medial femoral defects	**2.2 (1.3, 3.8)****	2.0 (1.0, 4.1)
Lateral tibial defects	**2.5 (1.5, 4.2)****	1.6 (0.8, 3.4)
Lateral femoral defects	**2.6 (1.6, 4.2)****	**3.7 (1.9, 7.3)****

Bold denotes a statistically significant result. *P < 0.05, **P < 0.01.
BMLs: bone marrow lesions; OR: odds ratio; 95% CI: 95% confidence interval.
†Adjusted for age, sex, body mass index and baseline site-specific defects if BMLs and site-specific BMLs if defects.
‡Further adjusted for meniscal extrusion and meniscal tear.

6.3.3 Cartilage volume loss

Site-specific Associations

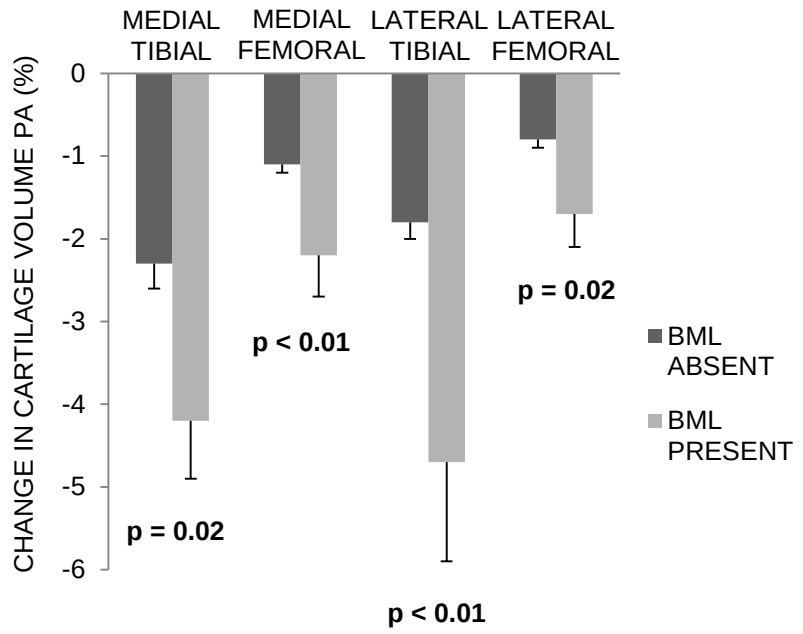
Figure 6.2 describes the univariate relationship between (A) baseline BMLs and (B) baseline cartilage defects with cartilage volume loss at each site. Cartilage volume loss was higher in those participants with a baseline BML (A). Those participants with a baseline cartilage defect score ≥2 lost significantly more cartilage at the medial and lateral tibial sites (B).

Table 6.3 describes the multivariable relationship between baseline BML and cartilage defect severity with change in cartilage volume. BMLs predicted site-specific cartilage volume loss at all four sites in a dose-response fashion. After further adjustment for meniscal damage this persisted at the medial femoral, lateral tibial, and lateral femoral sites. Cartilage defects predicted cartilage volume loss at the medial tibial site only; however, this did not persist after adjustment for meniscal damage. At the medial femoral site cartilage defects trended towards predicting cartilage volume loss ($P = 0.056$).

Figure 6.3 shows the interaction between baseline BMLs and cartilage defects on tibial cartilage volume loss. There was a higher rate of cartilage volume loss at both medial and lateral tibial sites when larger defects (grades 2–4) and BMLs (grades 2–3) were both present at the same site. There was no interaction between baseline BMLs and cartilage defects on femoral cartilage volume (data not shown).

Compartment-specific Associations

Although BMLs predicted site-specific cartilage volume loss, they did not predict compartment-specific cartilage volume loss at any site (data not shown). For example, medial femoral BMLs did not predict medial tibial cartilage volume loss.



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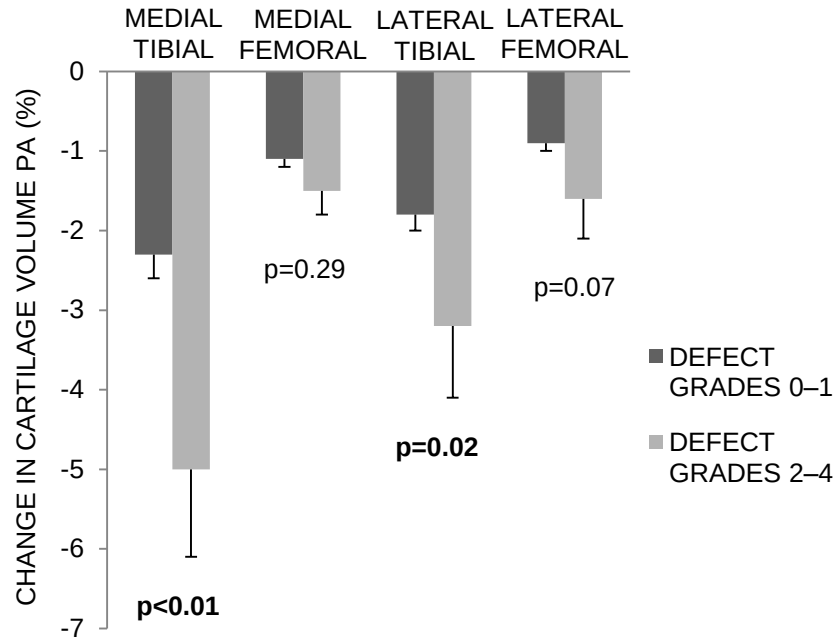


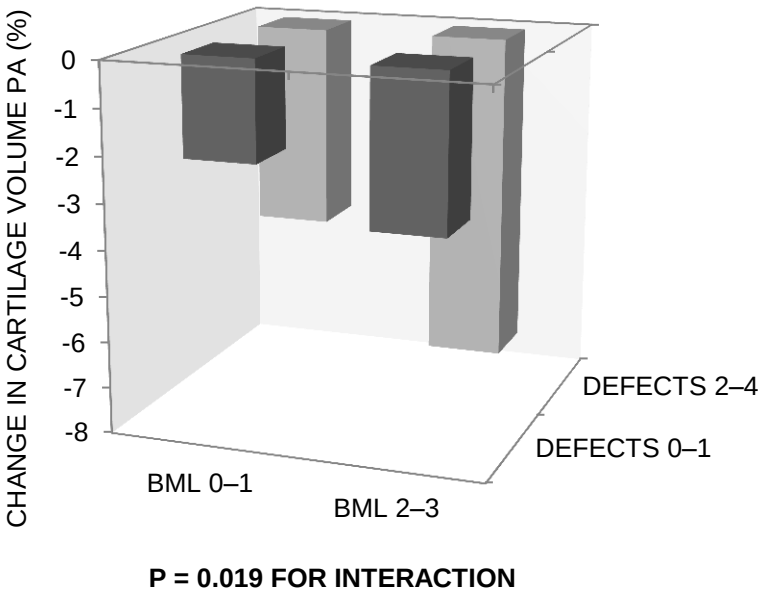
(B)

Figure 6.2. Baseline BMLs and cartilage defects with cartilage volume loss (% per annum). (A) Mean cartilage volume loss of participants with no BML at baseline versus those with a BML at baseline. (B) Mean cartilage volume loss of participants with baseline cartilage defect grades 0–1 versus those with baseline defect grades 2–4. Error bars represent standard error.

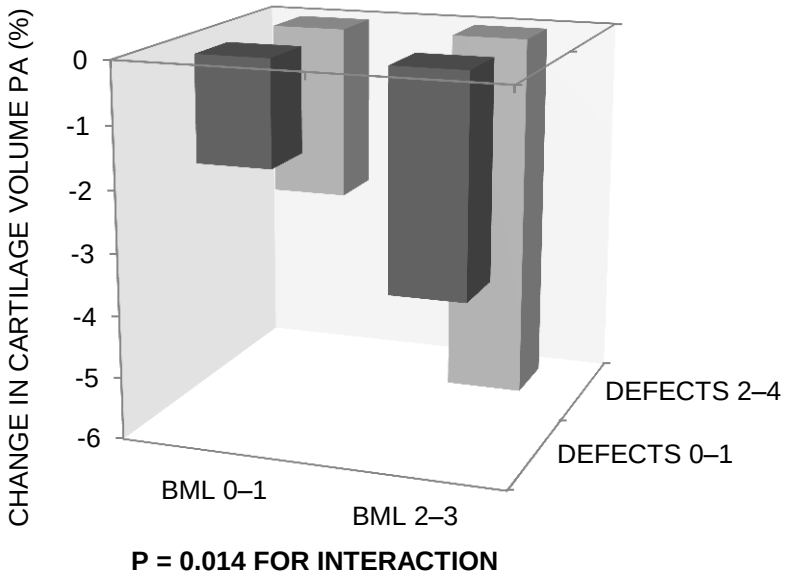
Table 6.3. Baseline BMLs (0–3) and baseline cartilage defects (0–4) predicting absolute changes in cartilage volume

	Multivariable β (95% CI)†	Multivariable β (95% CI)‡
Medial tibial		
BMLs	**-24.5 (-47.0, -2.0)***	-14.4 (-40.9, +12.1)
Cartilage defects	**-33.7 (-60.3, -7.1)***	-5.0 (-43.6, +33.7)
Medial femoral		
BMLs	**-42.0 (-63.6, -20.5)****	**-42.0 (-63.7, -20.4)****
Cartilage defects	-17.2 (-34.7, +0.4)	-17.2 (-34.8, +0.4)ϕ
Lateral tibial		
BMLs	**-35.2 (-56.1, -14.4)****	**-35.5 (-58.5, -12.6)****
Cartilage defects	-12.6 (-34.2, +9.0)	-21.7 (-50.2, +6.8)
Lateral femoral		
BMLs	**-22.1 (-39.5, -4.7)***	**-22.1 (-39.5, -4.7)***
Cartilage defects	-12.3 (-29.7, +5.1)	-12.3 (-29.7, +5.1)

Bold denotes a statistically significant result. *P < 0.05, **P < 0.01.
BMLs: bone marrow lesions; β: beta coefficient; 95% CI: 95% confidence interval.
†Adjusted for age, sex, body mass index, baseline site-specific cartilage volume and defects if BMLs and BMLs if defects.
‡Further adjusted for meniscal extrusion and meniscal tear.
ϕ P = 0.056



(A)



(B)

Figure 6.3. Interaction between baseline BMLs and baseline cartilage defects on tibial cartilage volume loss (% per annum). There was a significant interaction between (A) medial tibial BMLs and medial tibial cartilage defects; and (B) lateral tibial BMLs and lateral tibial cartilage defects, for site-specific cartilage volume loss.

6.3.4 Additional analysis

The results above were corroborated when BMLs were scored using the modified version of WORMS. Using the original scoring system BMLs predicted site-specific defect increases at all four sites (Table 6.2); whereas, using the WORMS system BMLs predicted site-specific defect increases at the medial femoral, lateral tibial, and lateral femoral sites (OR 2.9–13.7, all $P < 0.05$). Using the WORMS system BMLs predicted site-specific cartilage volume loss at the medial femoral, and lateral tibial sites ($\beta = -50.1$ to -122.1, all $P < 0.05$); whereas, using the original scoring system BMLs also predicted cartilage volume loss at the lateral femoral site (Table 6.3).

6.4 Discussion

This longitudinal study sheds light on the relationships between BMLs, cartilage defects, and cartilage volume loss. Baseline BMLs predicted site-specific cartilage defect progression and cartilage volume loss in a dose-response manner. To the best of our knowledge, this is the first study to show baseline cartilage defects predicted site-specific BML progression. Furthermore, there was an interaction between BMLs and cartilage defects on cartilage volume loss, with a much greater rate of tibial cartilage loss when both larger defects and BMLs were present at baseline.

Studies have only recently begun to examine the site-specific relationship between BMLs and cartilage changes (164, 166, 167). We have demonstrated a site-specific relationship between BMLs and both cartilage defect progression and a quantitative measure of cartilage volume loss. We found that BMLs predicted cartilage defect progression and cartilage volume loss at all four sites (medial tibial, medial femoral, lateral tibial, and lateral femoral). After further adjustment for meniscal extrusions and tears, BMLs continued to predict cartilage defect progression at all four sites and cartilage volume loss at the medial femoral, lateral tibial, and lateral femoral sites, demonstrating the associations presented are independent of meniscal damage. Importantly our results demonstrate a dose-response relationship exists between BMLs and site-specific cartilage damage and volume loss. For every unit increase in BML size, the odds of a cartilage defect progressing increased and more cartilage volume was lost over time. This is very similar to a recent study by Tanamas et al (184) which showed that the severity of BMLs was positively associated with the risk of knee joint replacement in subjects with well-established OA. Although our study included those with and without OA, it suggests that the size of the BML is important at different stages. However, we are unaware of any study which shows that BML size increases with stage of OA.

This study is unique in that it also explored whether BMLs at one site predicted cartilage damage or volume loss at another site. We observed only one compartmental association (medial femoral BMLs predicted medial tibial cartilage defect increases). The site-specific nature of most associations suggests BMLs may be having an effect on the cartilage directly adjacent to the BML. BMLs may precede cartilage damage by altering cartilage nutrition resulting in cartilage defects. Furthermore BMLs are made of a mix of cell infiltrates (157, 228) and possible cross-talk between subchondral bone and cartilage (229) could induce catabolism of the cartilage. However, it is also possible that BMLs may be a secondary phenomenon as a result of cartilage damage. Indeed, this is the first study to

demonstrate that baseline cartilage defects predicted site-specific BML progression. After further adjustment for meniscal damage this relationship was seen at the medial tibial and lateral femoral sites. Again we observed only one compartment association (lateral tibial defects predicted lateral femoral BML increases). Cartilage defects may exert an effect on the underlying bone by increased load transmission to the bone, resulting in BMLs. Alternatively, BMLs and cartilage defects may not necessarily drive one another, although it is possible. They may co-occur together in the pathway towards increased disease. Therefore, it remains unclear whether BMLs precede, accompany, or follow cartilage damage and volume loss in OA (164).

Previous studies have shown that cartilage defects predict cartilage loss (145, 146, 182). In this study, baseline cartilage defects predicted cartilage volume loss at the medial tibial site only; however, this did not persist after adjustment for meniscal damage. There was a trend towards cartilage defects predicting cartilage volume loss at the medial femoral site, independent of site-specific BMLs and meniscal damage. Baseline BMLs predicted cartilage volume loss at three out of the four sites, independent of site-specific defects and meniscal damage. This demonstrates that BMLs were better than cartilage defects at predicting cartilage volume loss. Additionally, there was an interaction between baseline cartilage defects and BMLs on tibial cartilage volume loss at the medial and lateral sites, with a much greater rate of tibial cartilage volume loss when both larger defects and BMLs were present at the same site. This supports a previous study, which used finite element modeling to examine the effect of osteochondral defects on the knee joint (230). They found that cartilage alterations were further exacerbated when bone damage was combined with base cartilage split and absence of vertical collagen fibrils (230).

Cartilage volume, cartilage defects, BMLs, and meniscal damage were all measured independently, which is a strength of this study. However this study has potential limitations as well. First, follow-up MRI scans were only available on a subsample of the full Tasmanian Older Adult Cohort (TASOAC) study. However, there were no significant differences between the subjects included in the current study and those in the rest of the cohort in regards to demographics, baseline cartilage defects, BMLs, and cartilage volume. Second, we used a study design with two time points to examine whether BMLs predicted cartilage defect progression and whether cartilage defects predicted BML progression. A study with more than two time points may give more insight into the causal pathways between BMLs and cartilage damage. Third, knee malalignment has been postulated as one factor explaining, at least in part, the association between BMLs and cartilage loss in OA (151, 165). However, in a previous study we found that baseline malalignment was not

associated with subsequent loss of cartilage volume or progression of chondral defects (231). Our current results suggest that malalignment may not be the driving factor, considering femoral BMLs did not predict tibial cartilage volume loss. If the effect of BMLs on cartilage volume loss was biomechanical, compartment-specific associations between BMLs and cartilage volume loss would be expected. However, because we did not have information about malalignment we cannot conclusively say whether or not malalignment plays a role in the associations we have seen. Fourth, cartilage defects were assessed on T1-weighted gradient-recalled echo (GRE) MR images and some research groups propose that GRE type sequences are less suited to detect cartilage defects; however, we have recently published a letter to the editor of *Arthritis & Rheumatism* to address this issue (see Appendix 4). There is evidence to demonstrate that GRE type sequences are accurate and reliable for detecting cartilage defects with high sensitivity and specificity compared to arthroscopic results (232-234). While our measure of cartilage defects may contain some measurement error and misclassification, it is likely to be random and would dilute the effects we see, thus reducing our ability to detect significant findings. Lastly, BMLs were read on T2-weighted images using a scoring system which is widely-published (134, 174, 235); however, we have been made aware that scoring BMLs based on how many slices they appear on may bias towards flat but shallow lesions. For this reason, we extended our observation and performed a separate analysis in which BMLs were also scored by a different research group using a modified version of the WORMS method on T1-weighted images. Reading BMLs on T1-weighted MRI sequences may result in a more conservative analysis; however, d'Anjou et al (236) recently published a letter to the editor of *Osteoarthritis and Cartilage* to address whether non-cystic BMLs can be accurately measured using GRE type sequences. The authors presented evidence to demonstrate that GRE type sequences are equally effective in detecting the presence of BMLs compared with T2-weighted fast spin echo sequences (236). The results of the current study using both scoring systems with the two sequence types were highly consistent providing reassurance that our findings are valid.

In conclusion, baseline BMLs predicted site-specific defect progression and cartilage volume loss in a dose-response manner, which suggests BMLs may have a local effect on cartilage homeostasis. Baseline cartilage defects predicted site-specific BML progression, which may represent increased bone loading adjacent to defects. These results suggest BMLs and cartilage defects are interconnected and play key roles in knee cartilage volume loss; thus, both should be considered targets for intervention.

Chapter 7 - Natural history and clinical significance of MRI-detected bone marrow lesions at the knee: a prospective study in community-dwelling older adults

7.1 Introduction

Osteoarthritis (OA) is a complex disease characterised by involvement of multiple tissues in the synovial joint. There is strong evidence that bone plays an important role in the pathogenesis of OA and it has been suggested that bone changes may precede cartilage damage (237). Recently we have shown that elevated tibial bone area and subchondral bone mineral density (sBMD) predicted cartilage defect increases (Chapter 5). Additionally, tibial bone area predicted cartilage volume loss. Bone marrow lesions (BMLs) have also been recognised as an important feature of knee OA (150, 151). They are associated with structural changes in the knee, including joint space loss on radiographs (151), cartilage defect progression (158) and cartilage loss on magnetic resonance (MR) images (158, 163, 165). BML histology is heterogeneous and includes a mix of pathological changes. Zanetti et al (152) found that BMLs in the knee in subjects with severe OA undergoing total knee replacement consisted of several abnormalities including bone marrow necrosis, abnormal trabeculae, bone marrow fibrosis, bone marrow bleeding, and bone marrow edema. BMLs have also been described in other rheumatic conditions such as rheumatoid arthritis (RA) (238), osteonecrosis (239), ankylosing spondylitis (240), and transient osteoporosis of the hip (241) and are often referred to as bone marrow edema (BME). In RA, it is suggested that BME represents cellular infiltrate within the subchondral bone (238) and is associated with painful and aggressive disease (242). Although BMLs in OA and BME in RA appear similar on MR images, it is unclear whether they are under the same pathological processes.

There is conflicting data on the natural history of BMLs in knee OA. Most studies have focused on symptomatic OA populations. One study reported that <1% of patients showed a BML decrease over 30 months (165) while, in contrast, another study found that 20% of BMLs decreased over 2 years (177). In subjects with prevalent knee OA or at risk for OA, Roemer et al (166) found that the majority (50%) of pre-existing BMLs decreased in size after 30 months follow-up. The reasons behind these variations are unclear.

A number of studies have linked BMLs with knee pain (81, 150, 175) although other studies have failed to demonstrate such a relationship (176-178). In pain-free populations, incident BMLs (175) and increases in BMLs (81) have been shown to be associated with development of knee pain. However, other studies in mostly OA subjects have reported no association between changes in BMLs and Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index pain scores at baseline (176), WOMAC scores after 2 years (177), or changes in WOMAC scores (178). Importantly, it

remains unknown whether reduction or resolution of BMLs is associated with improved knee pain. Furthermore, patients with OA experience stiffness and limited function; however, there is little data on the association between function, stiffness and BMLs.

Another important clinical outcome in knee OA is joint replacement surgery. It is well-established that radiographic severity and pain are strong predictors of joint replacement surgery (179, 180); however, there have been limited prospective studies examining structural factors and knee replacement surgery. In subjects with symptomatic knee OA, ultrasound detected effusion (181), articular cartilage defects (182), rate of tibial cartilage loss and tibial bone size (183) predicted knee joint replacement. A recent study by Tanamas et al (184) showed that the severity of BMLs was positively associated with the risk of knee joint replacement in subjects with well-established OA. It is unknown whether BMLs in a community-based sample also predict knee joint replacement.

The conflicting data on the natural history and clinical significance of BMLs may be due to studies grading BMLs semi-quantitatively, based on the extent of regional involvement. A truly quantitative measure of BML size may give more insight into actual changes over time. Therefore, this study aimed to: 1) describe the natural history of BMLs in a population-based sample using a quantitative measure; and 2) examine the clinical correlates of BMLs, including pain, function, and stiffness scores and total knee replacement surgery.

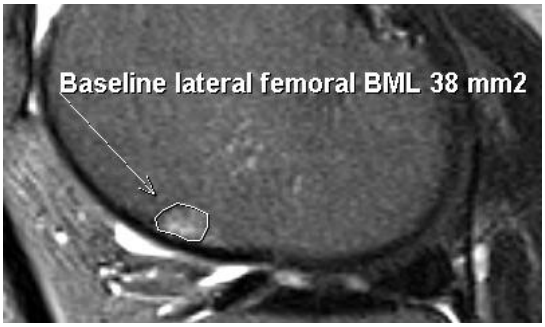
7.2 Materials and Methods

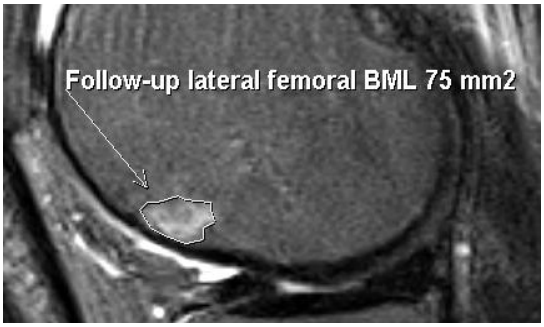
7.2.1 Subjects

A third follow-up of the Tasmanian Older Adult Cohort (TASOAC) study was recently completed. This was approximately a 5 year follow-up. This study utilises total knee replacement surgery data from both the 2.7 and 5 year follow-up.

7.2.2 Bone marrow lesions

BMLs were assessed using the areal scoring system as described in section 3.5.2 at baseline and approximately 2.7 years. Baseline and follow-up MRIs were read paired with the chronological order known to the observer and the observer blinded to clinical status. At baseline and the first follow-up, participants were given a BML score (mm^2) for each of the four sites (medial tibial, medial femoral, lateral tibial, and lateral femoral sites) as well as a total BML score, which was the sum of the scores at each site. Figure 7.1 illustrates a change in BML size from baseline to follow-up.





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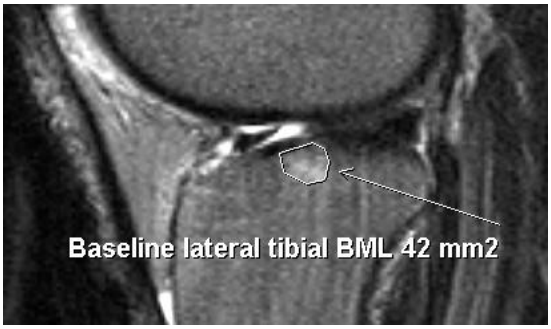

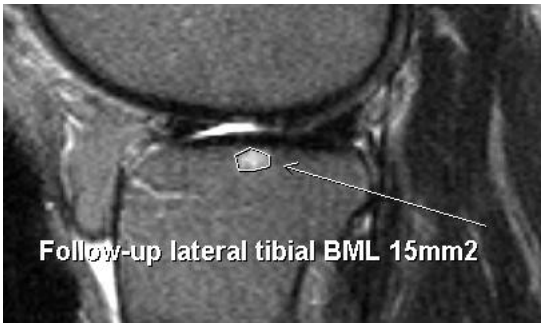

(B)

Figure 7.1. Change in BML size. (A) BML increase from baseline to follow-up. (B) BML decrease from baseline to follow-up.

7.2.3 WOMAC scores

Knee pain, function, and stiffness were assessed by self-administered questionnaire (WOMAC) (83), at baseline and the first follow-up (Appendix 5). WOMAC uses a 10-point scale from 0 (no pain, stiffness, or function deficit) to 9 (most severe pain, stiffness or severe function problems). Knee pain, function, and stiffness assessment consisted of 5, 17, and 2 questions each, therefore the range for each of these is from 0–45, 0–153, and 0–18, respectively.

In further analysis, knee pain was assessed using the five sub-scales, which included knee pain while walking on a flat surface, going up and down stairs, at night while in bed, sitting or lying, and standing upright. These ranged from 0–9.

Subjects also completed a questionnaire on medication use at baseline and the first follow-up.

7.2.4 Knee replacement surgery

Participants were asked whether they had undergone total knee replacement surgery since their first visit at the 2.7 and 5 year follow-ups. Although MRI scans were taken of the right knee only at baseline, replacement surgery data was collected for both knees.

7.2.5 Quality of life

The Assessment of Quality of Life (AQoL) instrument was used to measure health-related quality of life. The AQoL is a valid (243) measure of quality of life, with reliability in a population-based study of 0.81 (Cronbach's α) (244). Total AQoL score ranged from 0 (perfect health) to 45 (worst possible health state).

7.2.6 Statistical analysis

In order to examine the natural history of BMLs a significant change in BML size was defined as any change above (increase) or below (decrease) the least significant criterion (LSC) (245), which takes into account measurement error and the correlation between the BML measurements at baseline and follow-up. The formula was as follows:

$$LSC = 1.96 \times \sigma \sqrt{2\ 1-\rho}$$

where σ is the standard error of the mean and ρ is the serial correlation. LSC was calculated to be 25 mm^2 (where $\sigma = 11.67$ and $\rho = 0.3810$). Therefore an increase in

BML size was any change above 25 mm^2, which included new or progressing BMLs. A decrease in BML size was any decrease greater than 25 mm^2, which included resolved or regressing BMLs.

Logistic regression analysis was used to examine the association between baseline BMLs (absent versus present) and increases in BMLs (no increase or incident BML versus increase or incident BML) and demographic factors such as age, sex, and body mass index (BMI).

Mixed effects models were used to account for the correlated readings within an individual and examine the association between changes in WOMAC scores (pain, function, and stiffness) and continuous changes in BML size. Standard diagnostic checks of model adequacy and unusual observations were performed and revealed that some of the models were heteroscedastic. This is due to the fact that much of the data is clumped at zero because BMLs were measured at four separate sites and the majority of participants who had a BML present had it at only one of the four sites. To our knowledge there is no commercially available software to deal with data of this sort in longitudinal analysis. As a result we have performed two separate analyses examining, 1) BML size change at all four sites (medial tibial, medial femoral, lateral tibial, and lateral femoral); and 2) total BML size change (all four sites combined). This was done in order to check the consistency of our results. We also stratified the analysis by presence or absence of radiographic osteoarthritis (ROA), as the results were quite different for each sub-group.

Over the course of the study period (5 years), there were 12 knee replacements; therefore, we were only able to perform an exploratory analysis between BMLs and knee replacement surgery. Logistic regression and exact logistic regression modelling were used to examine whether baseline BMLs measured using the ordinal scale predicted knee replacement surgery after adjustment for potential confounders.

7.3 Results

7.3.1 Subjects

The current study consists of a sample of 395 participants (mean age 63 years, range 52–79 years, 51% female) who had MRI measures at baseline and the first follow-up. MRI scans were discontinued after this sample due to decommissioning of the MRI scanner. Additional data on knee replacement surgery at 5 years was also available on these subjects. At baseline, there were no significant differences in demographics, ROA, WOMAC function or stiffness scores, AQoL scores, or leg strength between the rest of the cohort (n=704) and the subjects included in the current study (n=395). There was a small difference in the WOMAC pain scores between the subjects in the current study [mean pain score 3.2 (standard deviation (SD) 6.3)] compared with the rest of the cohort [mean pain score 4.1 (SD 6.4); $P = 0.03$ for difference]. The characteristics of the study population are presented in Table 7.1.

Table 7.1. Characteristics of participants at baseline (n=395)

	Value*
Age (year)	63.2 (7.2)
Male sex (%)	49
Height (cm)	167.2 (8.8)
Weight (kg)	77.3 (14.2)
Body mass index (kg/m^2)	27.6 (4.5)
ROA present (%)	58
BML present (%)	43
Mean BML size (mm^2)	72.7 (74.6)
WOMAC	
Pain (0–45)	3.2 (6.3)
Function deficit (0–153)	10.4 (21.9)
Stiffness (0–18)	1.5 (2.9)
Leg strength (kg)	92.3 (47.5)
AQoL (0–29)	7.0 (5.0)

*Mean (standard deviation) except for percentages.
ROA, radiographic osteoarthritis; BML, bone marrow lesion; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; AQoL, Assessment of Quality of Life.

7.3.2 Natural history and demographic factors

At baseline, 43% of participants (n = 168/395) had one or more BML present at the medial tibial, medial femoral, lateral tibial, and/or lateral femoral site. 114 subjects had a BML at one site only, 43 had a BML at two sites, 10 had a BML at three sites, and 1 had a BML at all four sites. Therefore at all four sites combined, there were 234 total BMLs present at baseline.

The overall prevalence in those with (43%) and without ROA (41%) was similar; however, those with ROA had more total BMLs present (144) compared to those without ROA (80). In those with ROA, 58 subjects had a BML at one site only, 26 had a BML at two sites, 10 had a BML at three sites, and 1 had a BML at all four sites. Those without ROA had BMLs present at one or two sites only; 50 had a BML at one site and 15 had a BML at two sites.

Table 7.2 describes the association between baseline BMLs and increasing BMLs with baseline demographic factors. Those who had a BML present at baseline had a higher BMI and were more likely to be male. Males were also more likely to have a BML increase. Age or BMI did not predict BML increases.

Figure 7.2 describes the natural history of BMLs in the whole population and split by ROA. About half the lesions present at baseline remained stable, with similar proportions both worsening and improving. Those with ROA had higher odds of a BML increasing compared to those without ROA (odds ratio (OR) 2.2, $P = 0.017$). This was the only significant difference in the natural history between those with and without ROA.

Of those that did not have a BML at baseline (n = 227), 7% developed one or more BMLs from baseline to follow up. Incidence was also similar for those with (7.2%) and without ROA (6.5%).

Table 7.2. Relationship between baseline BMLs and increasing BMLs with baseline demographic factors*

	Univariate OR (95% CI)	*P*	Multivariable OR (95% CI)†	*P*
Absence/Presence of BML at baseline				
Age	1.00 (0.82, 1.22)	0.992	0.99 (0.80, 1.21)	0.905
Male sex	**1.61 (1.08, 2.41)**	**0.020**	**1.70 (1.13, 2.56)**	**0.011**
BMI	**1.31 (1.07, 1.61)**	**0.009**	**1.34 (1.09, 1.65)**	**0.005**
BML increase at any site**				
Age	1.10 (0.86, 1.40)	0.463	1.08 (0.84, 1.39)	0.529
Male sex	**1.74 (1.05, 2.86)**	**0.030**	**1.78 (1.07, 2.95)**	**0.026**
BMI	1.19 (0.94, 1.51)	0.146	1.23 (0.97, 1.58)	0.093

*Values are per 1 standard deviation increase in respective variable (except sex).
OR, odds ratio; 95% CI, 95% confidence interval; BMLs, bone marrow lesions; BMI, body mass index.
**No increase or incident BML versus an increase or incident BML at any site (medial tibial, medial femoral, lateral tibial, and/or lateral femoral)
†Adjusted for sex and BMI in the age model, age and BMI in the sex model, or age and sex in the BMI model.
Boldface denotes statistically significant result.

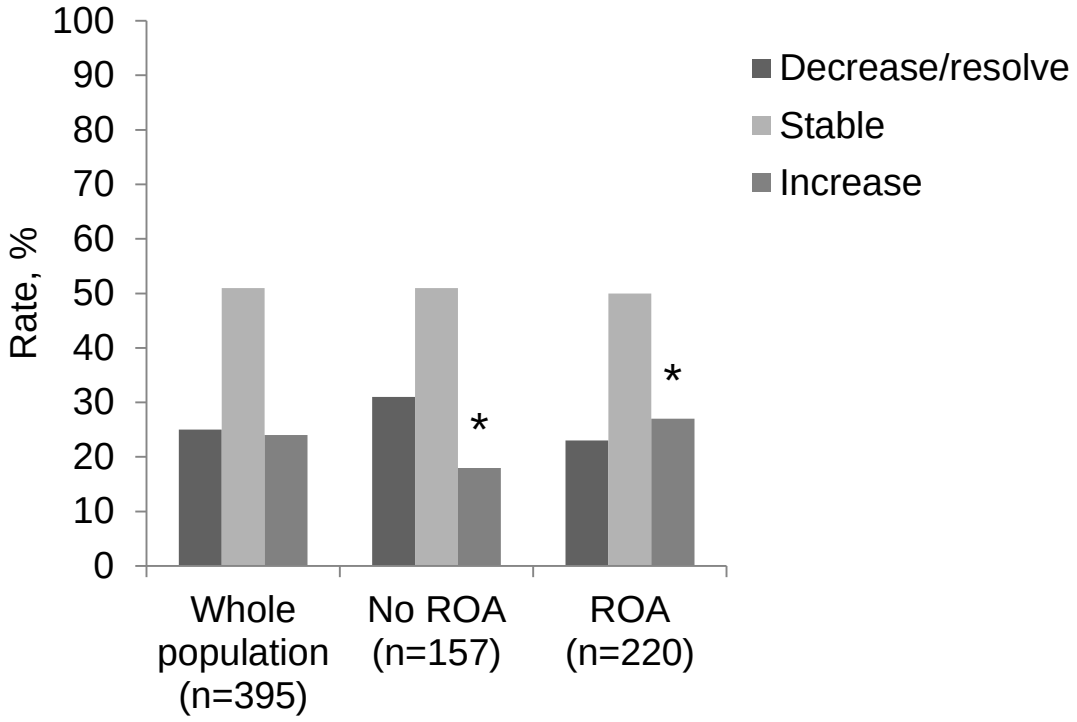

Figure 7.2. Natural history of BMLs. *Those with ROA had higher odds of a BML increasing compared to those without ROA (OR 2.2, *P* = 0.017).

7.3.3 WOMAC scores and BMLs

Table 7.3 describes the association between changes in WOMAC scores and changes in BML size, stratified by ROA. A change in knee pain and function was associated with a change in BML size at all four sites, but only in those participants without ROA. These results were also consistent when using change in total BML size (all four sites combined) as the independent factor. Importantly the association between change in function and change in BML size disappeared after further adjustment for change in pain ($\beta = 0.10–0.23$, $P > 0.05$), demonstrating that the association between changes in function and changes in BML size is mediated by changes in pain. In those without ROA, a one SD increase in total BML size led to a 1.13-unit increase in pain ($P = 0.009$). Similarly, a one SD decrease in total BML size led to a 1.13-unit decrease in pain ($P = 0.009$), in those without ROA. There were no associations between changes in pain, function, or stiffness and changes in BML size (at all four sites or total BML size) in those with ROA.

Table 7.4 describes the association between changes in the five WOMAC pain sub-scales and changes in BML size, stratified by ROA. Changes in knee pain when walking on a flat surface, going up and down stairs, and at night while in bed was associated with changes in BML size at all four sites, again only in those participants without ROA. These results were also consistent when using change in total BML size (all four sites combined) as the independent factor. There were no associations between changes in any of the five WOMAC pain sub-scales and changes in BML size (at all four sites or total BML size) in those with ROA.

Additional analyses in which we adjusted for baseline pain medication use or changes in pain medication did not alter our results, nor did separate adjustments for nonsteroidal anti-inflammatory drugs (NSAIDs). The results also remained unchanged after adjustment for tibial bone area and sBMD.

Table 7.3. Relationship between a change in WOMAC scores and a change in BML size stratified by ROA*

	BML size change at all 4 sites**				Total BML size change			
	Univariate β (95% CI)	*P*	Multivariable β (95% CI)†	*P*	Univariate β (95% CI)	*P*	Multivariable β (95% CI)†	*P*
No ROA								
Pain change	**0.57 (0.15, 0.99)**	**0.008**	**0.56 (0.19, 0.92)**	**0.003**	**1.06 (0.10, 2.03)**	**0.031**	**1.13 (0.28, 1.98)**	**0.009**
Function change	1.20 (-0.08, 2.47)	0.067	**1.25 (0.22, 2.28)**	**0.017**	2.23 (-0.71, 5.17)	0.136	**2.55 (0.14, 4.95)**	**0.038**
Stiffness change	-0.01 (-0.20, 0.18)	0.925	0.04 (-0.13, 0.20)	0.664	-0.02 (-0.46, 0.43)	0.947	0.09 (-0.29, 0.48)	0.641
ROA Present								
Pain change	0.07 (-0.29, 0.43)	0.715	0.03 (-0.28, 0.35)	0.844	0.11 (-0.54, 0.77)	0.733	0.06 (-0.53, 0.64)	0.848
Function change	0.16 (-0.77, 1.08)	0.740	0.13 (-0.62, 0.89)	0.729	0.26 (-1.41, 1.94)	0.756	0.23 (-1.17, 1.63)	0.750
Stiffness change	0.03 (-0.13, 0.19)	0.723	0.03 (-0.11, 0.17)	0.655	0.04 (-0.25, 0.34)	0.772	0.05 (-0.21, 0.30)	0.721

*Values are the change in pain, function, or stiffness score per 1 standard deviation change in BML size (mm^2).
BML, bone marrow lesion; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; ROA, radiographic osteoarthritis.
β, beta coefficients; 95% CI, 95% confidence interval.
**Medial tibial, medial femoral, lateral tibial, and lateral femoral.
†Adjusted for age, sex, body mass index, leg strength, quality of life, and baseline pain, function, or stiffness score depending on the model.
Boldface denotes statistically significant result.

Table 7.4. Relationship between changes in the five WOMAC pain sub-scales and changes in BML size stratified by ROA*

	BML size change at all 4 sites **				Total BML size change			
	Univariate β (95% CI)	*P*	Multivariable β (95% CI)†	*P*	Univariate β (95% CI)	*P*	Multivariable β (95% CI)†	*P*
No ROA								
Walking on a flat surface	**0.15 (0.05, 0.26)**	**0.005**	**0.14 (0.06, 0.22)**	**0.001**	**0.30 (0.05, 0.54)**	**0.018**	**0.29 (0.11, 0.48)**	**0.002**
Going up and down stairs	**0.16 (0.04, 0.28)**	**0.008**	**0.15 (0.05, 0.25)**	**0.003**	**0.33 (0.05, 0.60)**	**0.020**	**0.33 (0.09, 0.57)**	**0.006**
At night while in bed	**0.13 (0.03, 0.23)**	**0.014**	**0.11 (0.04, 0.18)**	**0.002**	0.22 (-0.01, 0.45)	0.066	**0.21 (0.04, 0.38)**	**0.014**
Sitting or lying	0.06 (-0.03, 0.15)	0.172	0.07 (-0.01, 0.15)	0.073	0.11 (-0.10, 0.31)	0.314	0.14 (-0.04, 0.33)	0.130
Standing upright	0.06 (-0.03, 0.15)	0.160	0.08 (-0.001, 0.16)	0.054	0.12 (-0.09, 0.33)	0.271	0.16 (-0.03, 0.35)	0.089
ROA present								
Walking on a flat surface	-0.02 (0.08, 0.05)	0.652	-0.02 (0.08, 0.05)	0.617	-0.02 (-0.15, 0.10)	0.686	-0.03 (-0.14, 0.09)	0.645
Going up and down stairs	0.02 (-0.07, 0.12)	0.660	0.02 (-0.06, 0.11)	0.601	0.03 (-0.14, 0.20)	0.722	0.03 (-0.12, 0.19)	0.673
At night while in bed	0.03 (-0.07, 0.14)	0.534	0.01 (-0.07, 0.09)	0.871	0.05 (-0.14, 0.25)	0.589	0.01 (-0.13, 0.16)	0.850
Sitting or lying	0.01 (-0.08, 0.10)	0.863	-0.01 (-0.08, 0.06)	0.827	0.01 (-0.15, 0.17)	0.895	-0.01 (-0.14, 0.12)	0.847
Standing upright	0.03 (-0.05, 0.12)	0.431	0.01 (-0.06, 0.07)	0.800	0.05 (-0.10, 0.21)	0.504	0.01 (-0.11, 0.14)	0.814

*Values are the change in pain sub-scale score per 1 standard deviation change in BML size (mm^2).
BML, bone marrow lesion; ROA, radiographic osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; β, beta coefficients; 95% CI, 95% confidence interval.
**Medial tibial, medial femoral, lateral tibial, and lateral femoral.
† Adjusted for age, sex, body mass index, leg strength, quality of life, and baseline pain sub-scale score depending on the model.
Boldface denotes statistically significant result.

7.3.4 Knee replacement surgery

There were 12 total knee replacements from baseline to the 5 year follow-up and baseline BML data assessed using the ordinal scale was available on all of these. The ordinal and areal BML measures were done by two separate readers and the correlation between the two was high (r = 0.79, $P < 0.001$).

Seventy-five percent (9/12) of participants who had a knee replacement had a BML at baseline. Table 7.5 examines the relationship between knee replacement surgery and baseline BMLs. An exploratory analysis revealed that in univariate analysis baseline BMLs in the right knee predicted incident knee replacement of the left, right, and right and left knee combined. Baseline BML severity of the right knee was a stronger predictor of a right knee replacement (OR 2.75/unit, $P < 0.01$); however, also predicted left knee replacement (OR 1.92/unit, $P < 0.01$).

In multivariable analysis, BML presence and severity predicted right and left knee replacement after adjustment for age and sex. A further adjusted model examining knee replacements of the right and left knee combined revealed that BML severity predicted knee replacement after adjustment for a large number of confounders (OR 2.10/unit, $P = 0.019$). A consistent trend to an association was observed for presence of any BML at baseline and knee replacement surgery of the right and left combined, but this did not achieve statistical significance in the adjusted model (OR 5.67, $P = 0.124$), although the OR did not change from the univariate analysis.

Table 7.5. Relationship between knee replacement surgery of the left, right, and right and left combined and baseline BMLs of the right knee*

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI) †	P value
Left knee replacement (n=7)				
BML severity (0–8)	**1.92 (1.40, 2.62)**	**<0.01**	**2.78 (1.58, 4.90)**	**<0.01†**
BML presence/absence	4.60 (0.88, 24.05)	0.07	**12.85 (1.82, 90.91)**	**0.011†**
Right knee replacement (n=8)				
BML severity (0–8)	**2.75 (1.81, 4.18)**	**<0.01**	**2.88 (1.84, 4.52)**	**<0.01†**
BML presence/absenceϕ	**20.75 (3.17, α)**	**<0.01**	**22.63 (3.72, α)**	**<0.01†**
Knee replacement right and left (n=12)				
BML severity (0–8)	**2.04 (1.55, 2.69)**	**<0.01**	**2.10 (1.13, 3.90)**	**0.019‡**
BML presence/absence	**5.67 (1.51, 21.32)**	**0.01**	5.67 (0.62, 51.77)	0.124‡

*No knee replacement versus a knee replacement and baseline BMLs (measured on the ordinal scale and ranged from 0–12, which was the sum of the BML scores at all four sites).
OR, odds ratio; 95% CI, 95% confidence interval; BMLs, bone marrow lesions.
ϕUsing exact logistic regression because all eight subjects who had a right knee replacement had a BML present.
†Adjusted for age and sex.
‡Further adjusted for body mass index, knee pain, leg strength, cartilage defects, tibial bone area, and radiographic osteoarthritis.
Boldface denotes statistically significant result.

7.4 Discussion

This longitudinal study describes the natural history and clinical significance of BMLs in a randomly selected population of older adults. While incidence rates were low, BMLs (assessed by measuring maximal area) were not static, with around half either worsening or improving over the study timeframe. Change in BML size was associated with changes in pain, but only in those without established ROA. In an exploratory analysis we also found that baseline BML severity independently predicted knee joint replacement surgery.

This is the first study to report the natural history of BMLs in a community-based sample. Many of the previous studies have been performed in symptomatic OA cohorts, or in asymptomatic cohorts, which are not generalisable to the older population. We found that 43% exhibited one or more BMLs at baseline. In those with ROA the prevalence was similar. This is lower than in studies of patients with symptomatic OA (57–82% (165, 176-178)). In the whole population, of the BMLs present at baseline, 49% showed a change in size, with similar proportions both worsening (24%) and improving (25%). Davies-Tuck et al (175) concluded that in a healthy, pain-free population BMLs develop at a slower rate than has been reported in OA populations, and that BMLs are more likely to resolve. Similarly we found that BMLs increase at a slower rate in those without ROA. However, there were no significant differences in the rate of decreasing/resolving BMLs between the two subgroups. We found that 8% and 14% of BMLs completely resolved in those with and without ROA, respectively. This is much lower than Davies-Tuck et al.'s (175) study in healthy asymptomatic subjects which reported that 46% resolved. In subjects with prevalent knee OA or at risk for OA, Roemer et al (166) reported that nearly 41% resolved. The conflicting data on the natural history of BMLs may be due to a combination of factors; including different BML grading systems amongst studies, the diversity within study samples, as well as the variation in study designs. We assessed BMLs by measuring the maximal area at baseline and follow-up. We then calculated whether there was an actual change in BML size from baseline to follow-up using the LSC (245), which takes into account measurement error and the correlation between measurements at baseline and follow-up. This formula provides a realistic and clinically relevant tool to identify detectable difference greater than that expected from measurement error.

In our study, the incidence of new BMLs in subjects who were BML free at baseline was low (7%). Most studies which report BML incidence have been performed in symptomatic OA cohorts (165, 177, 246). Hunter et al (165) reported that a new BML

developed in 20% of knees in a population with primary knee OA. Similarly, Kornaat et al (177) reported that BML incidence was 21% in patients with OA. In subjects with prevalent knee OA or at risk for OA, Roemer et al (166) reported an incidence of nearly 33%. Our incidence rate of 7% is lower than that being described in symptomatic populations; in fact, it is even lower to what was reported in asymptomatic subjects without clinical knee OA (14%) (175). One reason could be because this was a community-based sample. However, as we have seen, the natural history of BMLs in similar populations is quite variable. It is likely that multiple factors contribute to the development of BMLs. A recent study has demonstrated a possible influence of physical effects on BMLs. In a cross-sectional design, Stehling et al (247) found that the prevalence of bone marrow abnormalities increased with the level of physical activity. Our current study is the first to report BML incidence in a community-based sample and it may be that BMLs vary considerably in nature because they are a result of many contributing factors.

We have found the relationship between BMLs and a change in knee pain is different for those with and without ROA. A change in BML size was associated with changes in pain as assessed by WOMAC scores, only in those without ROA, even after adjustment for a large number of factors that have been linked to knee pain (248). A 1-unit change in pain score would require a 140 mm^2 increase or decrease in BML area. This novel finding suggests that fluctuating knee pain may be attributable to BMLs in those participants with early stage disease. One explanation for the differences between those with and without ROA could be that once the disease progresses, there is other structural pathology contributing to knee pain. Indeed, we did find that those with ROA were more likely to have a BML present at multiple sites, so perhaps a change in one BML may not result in a symptomatic change because of other BMLs present. To support this, a previous cross-sectional study from this cohort using the ordinal BML scores found that prevalence of knee pain increased with the number of sites BMLs were present on (174). This was independent of knee ROA. Other studies have also suggested that the size of BML is strongly related to knee pain (81, 150, 249). These studies included people with ROA; therefore, it is unclear why, in the current study, BML changes were not associated with pain changes in those with ROA. This finding will need to be confirmed in future studies.

Further analysis with the WOMAC pain sub-scales demonstrated consistent results in those without ROA. We found that changes in BML size was associated with changes in knee pain when walking on a flat surface, going up and down stairs, and at night while in bed.

Interestingly, to the best of our knowledge, this is the first study to demonstrate that a decrease in BML size was associated with an improvement in knee pain. This relationship was seen in those without ROA. There is increasing data to suggest that BMLs are reversible (166, 175, 177) and using an areal measure of BML size, we have found that a decrease is associated with a positive clinical outcome. This has important implications for intervention studies. Currently there are no disease-modifying osteoarthritis drugs (DMOADs) available to modify structural progression in OA; therefore, structure modification is now a primary aim in clinical drug trials. We believe there is increasing evidence to suggest that BMLs are a promising target. BMLs predict important disease outcomes such as cartilage loss and knee replacement, have the potential to regress and resolve, and are strongly linked to knee pain. Therefore by targeting BMLs it may be possible to slow disease progression as well as reduce pain in patients with OA. BMLs are visualised using standard fluid-sensitive sequences; however, new advanced imaging analysis techniques (such as T1rho and T2 relaxation time quantification, and delayed gadolinium-enhanced MRI of cartilage (dGEMRIC)) have been developed. dGEMRIC measures glycosaminoglycan (GAG) concentrations in articular cartilage and GAG content can change quickly, therefore dGEMRIC can be used to determine if altering BML natural history improves cartilage biochemistry. There is no doubt that both standard and advanced MRI techniques will play an important role in guiding future treatments in OA.

Our exploratory analysis of the relationship between BMLs and knee replacement surgery revealed that baseline BML severity independently predicted knee replacement both before and after adjustment for confounding factors. The estimate changed little after adjustment for pain severity and radiographic change, indicating that the effect of BMLs on joint replacement was not mediated through these well-established drivers of joint replacement (179, 180). This suggests that BMLs may themselves be a marker of fast progression and this in turn could explain why BMLs of the right knee predicted both right and left knee replacements. However, in view of the small numbers of knee replacement, these results need to be interpreted with caution and require confirmation in larger studies.

We have previously published data on the demographic associations with BMLs. In a separate study we showed that BMLs were more common with increasing age, male sex, and increasing BMI (235). In the current study we did not find an association between age and BMLs; however, male sex remained a predictor of BML increases. It is unclear why males are more likely to develop BMLs. It is possible this is linked to knee trauma and knee injuries but systemic and metabolic factors may also play a role. Identifying risk factors and biomarkers for disease outcomes such as BMLs is important as it might shed

light on the pathogenesis of OA. It is now understood that BMLs are an important feature in OA; however, further work is required to identify a more complete set of risk factors which should include both demographic, environment, and lifestyle factors, combined with MRI biomarkers.

This study does have potential limitations. First, for the current study, 704 were not included due to decommissioning of the MRI scanner at follow-up. There were no significant differences between those studied and the rest of the cohort in regards to demographics, ROA, WOMAC function or stiffness scores, AQoL scores, or leg strength. However, those studied had a modestly lower WOMAC pain score. Second, as previously mentioned, the numbers of knee replacement were limited. While we were able to adjust for many confounding factors in the knee replacement analysis, we had insufficient data on tibial cartilage loss to adjust for this, as we only had follow-up cartilage volume data on three out of the 12 subjects who underwent knee replacement surgery. We also did not have data on effusion which has been shown to predict knee replacement (181). Lastly, BML area was measured from the slice with the greatest BML size. This may bias towards shallow but flat lesions; however, it is customary to measure BMLs this way. The majority of previous studies also grade BMLs on the slice with the greatest BML size; however, they use a semi-quantitative scale (0–3) rather than an areal measure. We acknowledge that our measure of BMLs is only a surrogate measure of volume. Recent methods have been developed to measure BML volume using a autoregression model, as well as BML signal intensity (250, 251). It is our view that the slice thickness (4 mm) and interslice gap (0.5–1.0 mm) of our imaging protocol was too large to estimate volume with sufficient accuracy. Both the slice thickness and the interslice gap are likely to impact on the areal BML measurements. It is possible that a shallow BML may not be detected if it lies within the interslice gap. Also some lesions may be underestimated depending on where the slice has been taken. Smaller slice thicknesses allow for more slices to be taken and thus help to reduce measurement error. Although we acknowledge these factors as limitations it is important to consider the effect sizes we have shown. A 140 mm^2 change in BML areal size led to a 1-unit change in pain. This change is greater than what would be expected due to measurement error alone, as our calculated LSC was 25 mm^2.

In conclusion, BMLs (assessed by measuring maximal area) were not static, with similar proportions both worsening and improving in this population-based sample. A change in BML size was associated with changes in pain in those without ROA. This finding suggests that fluctuating knee pain may be attributable to BMLs in those participants with early stage disease. Baseline BMLs also predicted knee replacement

surgery. These findings suggest therapeutic interventions aimed at altering the natural history of BMLs should be considered.

Chapter 8 - A longitudinal study of the association between dietary factors, serum lipids, and bone marrow lesions of the knee

8.1 Introduction

Osteoarthritis (OA) is a whole organ disease characterised by gradual loss of articular cartilage. There is strong evidence that bone plays an important role in the pathogenesis of OA and it has been suggested that bone changes may precede cartilage damage (237). Bone marrow lesions (BMLs), visible using magnetic resonance imaging (MRI), have been recognised as a clinically important feature in OA (150, 151). A number of studies have linked BMLs with knee pain (81, 150, 174) (Chapter 7). They are also associated with many structural changes in the knee such as cartilage defect progression (158) (Chapter 6) and cartilage loss (158, 163, 165) (Chapter 6) on MR images and predict joint replacement surgery (184) (Chapter 7).

There is growing evidence implicating nutritional factors in OA (185). Specifically, nutrient and dietary supplements have been shown to be effective in relieving OA symptoms and some may play a role in the course of the disease (186). Elevated levels of fat and n-6 polyunsaturated fatty acids have been found in OA bone (187); whereas n-3 polyunsaturated fatty acids have been shown to modulate catabolic factors in articular cartilage destruction (188). There is limited data on the relationship between fatty acids and BMLs. Wang et al (189) reported that higher intakes of monounsaturated, total, and n-6 polyunsaturated fatty acids were associated with BMLs cross-sectionally. In a recent longitudinal design, they showed that increased saturated fat intake was associated with incident BMLs (190). These results need confirmation in different settings. Furthermore, it is unknown whether intakes of other dietary components such as total energy, protein, carbohydrate, and/or sugar are associated with BMLs.

Research has shown that the prevalence of vascular disease is high amongst people with OA (187, 191). Evidence suggests that these diseases may share risk factors, such as obesity, hypertension, high low-density lipoprotein (LDL) levels, elevated total cholesterol, diabetes, smoking, and diet (187, 191-194). Vascular pathology may contribute to the development of OA through its effects on the subchondral bone. Blood flow through the small vessels in the subchondral bone may be reduced by venous occlusion, which results in impaired venous circulation underlying the cartilage plate, joint hypertension, hypercoagulability and/or microemboli (192). These may result in subchondral bone ischemia, which can contribute to decreased nutrient supply to the overlying cartilage plate (192). Subchondral bone ischemia can also affect osteocyte death, leading to bone resorption, reducing the viability of subchondral bone (192, 195). BML histology is heterogeneous and includes osteonecrosis, edema, trabecular abnormalities and

bone remodelling (152). Additional MRI-histologic correlation studies of these lesions have demonstrated fat cell destruction and fibrovascular regeneration in the lesion area (155), as well bone marrow fibrosis in well-defined subchondral zones of OA (156). Recently Hunter et al (157) demonstrated that BMLs are sclerotic compared with unaffected regions from the same individual based on the increased bone volume fraction and increased trabecular thickness. Also, BMLs have been linked to ischemia and/or reperfusion injury (195, 196). Therefore it is possible that vascular pathology may influence BML development. To our knowledge there has been only one study which examined serum lipids and BMLs, reporting that serum cholesterol and triglyceride levels were associated with an increased incidence of BMLs (197). However, this study was conducted in asymptomatic women; therefore, further studies are needed in different populations to confirm this finding. Additionally is unknown whether serum lipids are associated with BML progression.

The aim of this study, therefore, was to describe the association between dietary factors, serum lipids and BMLs in a population-based sample of older adults.

8.2 Materials and Methods

8.2.1 Questionnaire

Self-report of smoking status, statin use, and disease status such as cardiovascular disease and diabetes were recorded by questionnaire.

8.2.2 Bone marrow lesions

BMLs were assessed using the areal scoring system as described in section 3.5.2 at baseline and approximately 2.7 years. Baseline and follow-up MRIs were read paired with the chronological order known to the observer and the observer blinded to clinical status. At baseline and the first follow-up, participants were given a BML score (mm^2) for each of the four sites (medial tibial, medial femoral, lateral tibial, and lateral femoral sites) as well as a total BML score, which was the sum of the scores at each site. Figure 7.1 illustrates a change in BML size from baseline to follow-up.

8.2.3 Dietary factors

Baseline dietary information was collected with the use of a self-administered, 74-item validated food frequency questionnaire (FFQ) that was developed specifically for use in Australian adults (211, 252). Ten possible frequency responses were available for each food item, ranging from "never or less than once per month" to "three or more times per day." This information was used to compute specific nutrient intakes such as total energy (kJ/day), fatty acid [total fats, monounsaturated, polyunsaturated, and saturated (g/day)], carbohydrate (g/day), protein (g/day), and sugar (g/day). An example of the output is provided in Appendix 3.

Information regarding over the counter medication was collected, which included information about vitamin and mineral supplementation and natural and herbal medications. We have chosen not to include these as part of nutrient intake in the current study because supplements have become more complex, with different brands containing highly variable ingredient combinations. As a result, evidence now suggests that brief and simple questionnaires (those which have been used in the current study) do not accurately reflect supplement intake and more validated methods are necessary (253). Therefore we have limited our definition of nutrient intake to that data obtained from the FFQ.

8.2.4 Serum lipids

Blood samples were collected at baseline after a 12-hour overnight fast, and assays were conducted to enzymatically measure total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides using an Olympus AU5400 automated analyser. The concentration of LDL cholesterol was calculated using the Friedewald formula (254). Assays were performed on thawed blood samples.

8.2.5 Statistical analysis

Logistic regression was used to examine the associations between baseline BMLs (absent versus present) with baseline dietary factors and lipids after adjustment for age, sex, body mass index (BMI), smoking, cardiovascular diseases, diabetes, radiographic osteoarthritis (ROA), and statin use in the lipids model. Each dietary factor was entered into a separate multivariable model adjusted for the appropriate covariates.

Mixed effect models were used to examine the association between change in total BML area and baseline dietary factors and lipids after adjustment for age, sex, BMI, baseline BMLs, smoking, cardiovascular diseases, diabetes, ROA, and statin use in the lipids model.

In order to compare with previous studies, a separate analysis was performed which examined only incident BMLs with baseline dietary factors and lipids. As we used a continuous, quantitative measure of BML size, we defined incident BMLs based on the least significant criterion (LSC) (245). As described in Chapter 7, the LSC was used to define significant changes in BML size when assessing BMLs as a continuous measure. The LSC takes into account measurement error and the correlation between the BML measurements at baseline and follow-up. The formula was as follows:

$$LSC = 1.96 \times \sigma \sqrt{2\ 1 - \rho}$$

where σ is the standard error of the mean and ρ is the serial correlation. LSC was calculated to be 25 mm^2 (where $\sigma = 11.67$ and $\rho = 0.38$). Therefore incident BMLs were defined as any new BML in those with no BMLs at baseline, which was larger than 25 mm^2. Logistic regression analysis was performed to examine incident BMLs with dietary factors and lipids. Standard diagnostic checks of model adequacy and unusual observations were performed. Hosmer-Lemeshow tests were performed to assess goodness-of-fit for the logistic regression models. The result for each logistic regression was > 0.05, indicating the model fit was adequate.

8.3 Results

8.3.1 Subjects

The current study consists of a sample of 394 participants (mean age 63 years, range 52–79 years, 50% female) who had MRI measures at baseline and follow-up (approximately 2.7 years). MRI scans were discontinued after this sample due to decommissioning of the MRI scanner. There were no significant differences in demographics, baseline energy intake, fatty acid intake, carbohydrate intake, protein intake, sugar intake, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, ROA, or statin use between the rest of the cohort (n=705) and the subjects included in the current study (n=394). The characteristics of the study population are presented in Table 8.1. Those participants with a BML present at baseline (n=168) were more likely to be male, had a higher BMI, and lower total and HDL cholesterol.

Table 8.1. Characteristics of participants according to presence or absence of BMLs at baseline*

	BML absent (n = 226)	BML present (n = 168)	*P*
Age (year)	63.2 (7.3)	63.2 (7.2)	0.99
Female sex (%)	56	44	**0.02**
BMI (kg/m^2)	27.1 (4.0)	28.3 (5.0)	**0.01**
Current smokers (%)	13	8	0.13
Cardiovascular disease (%)	4	9	0.07
Diabetes (%)	8	6	0.36
Statin use (%)	17	20	0.45
ROA present (%)	57	59	0.70
Dietary Factors			
Energy Intake (kJ/day)	7512 (2378)	7800 (2941)	0.30
Total fat (g/day)	71.5 (25.9)	74.2 (34.5)	0.40
Monounsaturated fat (g/day)	24.9 (9.8)	25.7 (12.8)	0.50
Polyunsaturated fat (g/day)	11.8 (5.0)	11.6 (6.1)	0.67
Saturated fat (g/day)	28.6 (12.3)	30.4 (15.2)	0.19
Total carbohydrates (g/day)	205.1 (67.8)	214.3 (76.1)	0.21
Total protein (g/day)	84.7 (31.4)	87.2 (43.1)	0.52
Total sugar (g/day)	95.6 (36.3)	100.5 (42.0)	0.22
Lipids			
Total cholesterol (mmol/L)	5.7 (1.1)	5.4 (1.1)	**0.01**
Triglycerides (mmol/L)	1.5 (0.9)	1.5 (0.8)	0.99
LDL cholesterol (mmol/L)	3.6 (0.9)	3.5 (1.0)	0.10
HDL cholesterol (mmol/L)	1.4 (0.4)	1.3 (0.3)	**0.01**

*Mean (standard deviation) except for percentages. *P* values determined by t-test or chi-square test (where appropriate).
Boldface denotes a statistically significant result.
BMLs, bone marrow lesions; BMI, body mass index; ROA, radiographic osteoarthritis; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
ROA was defined as any score ≥1 for joint space narrowing or osteophytes.

8.3.2 Cross-sectional

Table 8.2 documents the associations between baseline BMLs with dietary factors and lipids. Dietary factors were not significantly associated with baseline BMLs. In univariate analysis, total and HDL cholesterol were negatively associated with baseline BMLs. These results were not significant after adjustment for age, sex, BMI, smoking, cardiovascular diseases, diabetes, ROA and statin use. Statin use was not associated with baseline BMLs (odds ratio (OR) 1.22, $P = 0.452$); however, those on statin medication had a significantly lower total cholesterol level (OR 0.51, $P < 0.001$), higher triglyceride level (OR 1.64, $P < 0.001$), and lower LDL cholesterol level (OR 0.23, $P < 0.001$); therefore we adjusted for statin use in the multivariable analysis.

Table 8.2. The association between baseline BMLs, dietary factors and lipids*

	Univariate		Multivariable†	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Dietary Factors				
Energy intake	1.12 (0.91, 1.36)	0.285	1.07 (0.84, 1.35)	0.592
Total fat	1.09 (0.89, 1.33)	0.385	1.06 (0.84, 1.34)	0.630
Carbohydrate	1.14 (0.93, 1.39)	0.206	1.08 (0.85, 1.36)	0.525
Protein	1.07 (0.88, 1.30)	0.517	1.03 (0.82, 1.29)	0.785
Sugars	1.13 (0.93, 1.39)	0.217	1.11 (0.89, 1.39)	0.344
Individual Fats				
Monounsaturated fat	1.07 (0.88, 1.31)	0.502	1.02 (0.81, 1.29)	0.849
Polyunsaturated fat	0.96 (0.78, 1.17)	0.672	0.92 (0.73, 1.15)	0.455
Saturated fat	1.14 (0.94, 1.40)	0.187	1.14 (0.90, 1.44)	0.283
Lipids				
Total cholesterol	**0.76 (0.61, 0.95)**	**0.015**	0.81 (0.63, 1.02)	0.074‡
Triglycerides	1.00 (0.82, 1.22)	0.996	0.90 (0.72, 1.13)	0.384‡
LDL cholesterol	0.84 (0.68, 1.03)	0.101	0.88 (0.69, 1.11)	0.280‡
HDL cholesterol	**0.77 (0.62, 0.95)**	**0.016**	0.89 (0.70, 1.14)	0.357‡

*BML absent (226) versus present (168).
Odds ratios (OR) have been standardised.
†Adjusted for age, sex, body mass index, smoking, cardiovascular diseases, diabetes, and radiographic osteoarthritis.
‡Further adjusted for statin use.
Boldface denotes a statistically significant result.
BMLs, bone marrow lesions: 95% CI, 95% confidence interval; *P*, *P* value, LDL, low-density lipoprotein; HDL, high-density lipoprotein.

8.3.3 BML change

The association between baseline dietary factors and lipids with change in total BML size is presented in Table 8.3. Change in BMLs was positively associated with energy, carbohydrate, and sugar intake in a multivariable model adjusting for age, sex, BMI, baseline BMLs, smoking, cardiovascular diseases, diabetes and ROA. There were no associations between total fat intake or individual fats (monounsaturated, polyunsaturated, and saturated fat) and BML change. After adjustment for age, sex, BMI, baseline BMLs, smoking, cardiovascular diseases, diabetes, ROA and statin use, HDL cholesterol was negatively associated with change in BMLs.

There were no differences in the relationship between dietary factors and lipids with change in BMLs in those with and without ROA, as no interaction terms with ROA were significant. Additionally, similar results were seen when area change at all four sites (medial tibial, medial femoral, lateral tibial, and lateral femoral) was used instead of total area change.

Table 8.3. The association between dietary factors and lipids with a change in total BML size*

	Univariate		Multivariable†	
	β (95% CI)	*P*	β (95% CI)	*P*
Dietary Factors				
Energy intake	8.60 (-2.46, 19.67)	0.127	**13.57 (0.50, 26.64)**	**0.042**
Total fat	1.86 (-9.24, 12.95)	0.742	5.31 (-7.84, 18.46)	0.428
Carbohydrate	**14.45 (3.45, 25.46)**	**0.010**	**19.13 (6.33, 31.93)**	**0.004**
Protein	5.32 (-5.76, 16.41)	0.346	9.22 (-3.36, 21.80)	0.150
Sugars	**14.53 (3.52, 25.53)**	**0.010**	**16.91 (4.61, 29.21)**	**0.007**
Individual Fats				
Monounsaturated fat	3.37 (-7.73, 14.46)	0.551	6.79 (-6.24, 19.82)	0.306
Polyunsaturated fat	6.26 (-4.83, 17.34)	0.268	8.73 (-3.57, 21.03)	0.164
Saturated fat	-1.88 (-12.97, 9.22)	0.740	0.56 (-12.53, 13.64)	0.933
Lipids				
Total cholesterol	-5.45 (-16.62, 5.72)	0.338	-7.09 (-19.63, 5.45)	0.267‡
Triglycerides	-7.24 (-18.42, 3.95)	0.204	-5.99 (-18.46, 6.48)	0.346‡
LDL cholesterol	1.62 (-9.59, 12.83)	0.776	-1.12 (-14.19, 11.95)	0.866‡
HDL cholesterol	**-11.18 (-22.30, -0.05)**	**0.049**	**-13.48 (-26.65, -0.32)**	**0.045‡**

*Values are a change in total BML size (mm^2) per 1 standard deviation increase in dietary or lipid factor.
†Adjusted for age, sex, body mass index, baseline BMLs, smoking, cardiovascular disease, diabetes and radiographic osteoarthritis.
‡Further adjusted for statin use.
Boldface denotes statistically significant result.
BML, bone marrow lesion; β, beta coefficients; 95% CI, 95% confidence interval; *P*, *P* value; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

8.3.4 Incident BMLs

The relationship between baseline dietary factors and lipids and incident BMLs is presented in Table 8.4. There were 14 incident BMLs across all four sites (medial tibial, medial femoral, lateral tibial, and lateral femoral). Total fat intake was protective against incident BMLs after adjustment for age, sex, and BMI. Saturated fat was also protective against incident BMLs in a multivariable model. In regards to serum lipids, HDL cholesterol was protective against incident BMLs after adjustment for age, sex, BMI and statin use.

Table 8.4. The association between baseline dietary factors and lipids with incident BMLs*

	Univariate		Multivariable†	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Dietary Factors				
Energy intake	0.68 (0.34, 1.37)	0.282	0.64 (0.30, 1.38)	0.253
Total fat	**0.40 (0.17, 0.93)**	**0.034**	**0.33 (0.13, 0.86)**	**0.023**
Carbohydrate	0.96 (0.53, 1.71)	0.877	0.95 (0.51, 1.76)	0.860
Protein	0.79 (0.38, 1.67)	0.542	0.79 (0.36, 1.75)	0.560
Sugars	0.98 (0.54, 1.75)	0.933	0.95 (0.52, 1.74)	0.874
Individual Fats				
Monounsaturated fat	0.49 (0.22, 1.09)	0.080	0.42 (0.17, 1.05)	0.063
Polyunsaturated fat	0.82 (0.44, 1.52)	0.523	0.82 (0.43, 1.56)	0.553
Saturated fat	**0.29 (0.11, 0.78)**	**0.014**	**0.24 (0.08, 0.70)**	**0.009**
Lipids				
Total cholesterol	0.81 (0.44, 1.46)	0.479	0.68 (0.35, 1.34)	0.267‡
Triglycerides	0.69 (0.33, 1.45)	0.329	0.75 (0.34, 1.67)	0.477‡
LDL cholesterol	1.18 (0.67, 2.07)	0.570	1.03 (0.55, 1.92)	0.922‡
HDL cholesterol	0.52 (0.27, 1.03)	0.060	**0.34 (0.15, 0.79)**	**0.012**‡

*No BML present at baseline and follow-up (n=212) versus incident BML (n=14) at any site (medial tibial, medial femoral, lateral tibial, and lateral femoral).
Odds ratios (OR) have been standardised.
†Adjusted for age, sex, body mass index.
‡Further adjusted for statin use.
Boldface denotes a statistically significant result.
BML, bone marrow lesion; 95% CI, 95% confidence interval; *P*, *P* value; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

8.3.5 Additional analysis

Additional data on cartilage defects and meniscal pathology were available in this study. The dietary factors found to be associated with BML changes (energy, carbohydrate, and sugar intake) were not associated with cartilage defects, meniscal extrusion, or meniscal tears cross-sectionally or longitudinally. HDL cholesterol was also not associated with cartilage defects, meniscal extrusion, or meniscal tears cross-sectionally or longitudinally.

Information was also available about physical activity. After further adjustment for steps/day, measured by pedometers, the results were largely unchanged.

8.4 Discussion

This longitudinal study reports associations between dietary factors, serum lipids and BMLs. Despite no cross-sectional associations, baseline energy, carbohydrate and sugar intake predicted change in BMLs while HDL cholesterol was protective against BML change.

BMLs are associated with malalignment (151, 165), increased loading (255), and increased body weight (175, 256), emphasising the relationship between mechanical loading and BMLs. However, recent studies have suggested associations between BMLs and nutritional factors (189, 190) suggesting a systemic role in BML pathology. We found that certain dietary factors (specifically total energy, carbohydrate, and sugar intake) were associated with BML change. Specifically, a higher total energy, carbohydrate, and sugar intake was associated with BML development and progression. This suggests that an increase in these dietary factors may be detrimental to BMLs. Carbohydrate and sugar intake could be surrogate measures for increasing energy, and it seems most likely that increased energy is the major contributor to BMLs. Evidence shows that a high energy diet increases free radical production and oxidative stress (257, 258), which could be having an effect on BMLs. Additionally the association we found was on a continuous scale, therefore decreased energy, carbohydrate, and sugar intake was associated with BML decreases. Relating to this there is also evidence showing that caloric restriction has anti-oxidative and anti-inflammatory vasoprotective effects in both animal and human studies (259, 260). Therefore there may be a role for dietary modification to alter BML natural history. However, the relationship we report between dietary factors and BMLs may also not be causal, and it may be that factors associated with diet are the underlying cause (e.g. physical activity). In our study we also collected information about physical activity levels in the form of steps/day measured by pedometers for two weeks a year. This is a good reflection of usual physical activity (201, 202). When we further adjusted for steps/day in the analyses the results were largely unchanged, suggesting the association is independent of physical activity. However, it is possible that there are other unknown confounders.

In order to compare to previous studies, we did a separate analysis examining only incident BMLs. Total fat and saturated fat intake was protective against incident BMLs. These findings were unexpected and inconsistent. We found no associations between fat and BMLs cross-sectionally (Table 8.2) or in the longitudinal analysis (Table 8.3) in which we used a continuous measure of BML change. Traditionally, longitudinal studies are

superior for demonstrating causal relationships; however, our incident results also conflict with a recent longitudinal study by Wang et al (190) which showed that increased saturated fat was associated with incident BMLs. These differences could reflect multiple comparisons in our current study. As each dietary factor was entered into a separate multivariable model, this study contains 24 dietary comparisons with BMLs. Simple techniques such as the Bonferroni method can be used to adjust for multiple comparisons; however, this can be very conservative (261). Therefore, it is our standard practice not to adjust for multiple comparisons but to present all comparisons in the paper so that the reader can assess this. However, this can increase the rate of false positive results (Type I error). As this study is one of the first to examine diet and incident BMLs, further studies are need to confirm our finding that fat is protective against incident BMLs.

Alternatively the discrepancy may reflect differences amongst study samples. In the study by Wang et al (190) participants included only those without clinical signs of knee OA; therefore, no ROA was present and they were pain free. Our current study was a population-based sample and included those with and without clinical knee OA. Ideally, we would have liked to perform a sub-group analysis based on ROA and knee pain, in order to better compare to the previous study; however we only had 14 incident BMLs and this was not possible. Furthermore, the collection of dietary intake data in the study by Wang et al (190) occurred 10–14 years prior to the study period rather than immediately before. It is possible that significant alterations in dietary behaviour occurred within this time frame; however, there is some evidence that nutrient intake is relatively stable and tends to be more stable with increasing age (262, 263). It seems logical that fat intake may be detrimental to BML development, given the histology of BMLs and the recent evidence linking them to vascular disease (197). Therefore our finding that fat is protective is surprising and further work should be done to explore fat intake with BML development and progression. Specifically, now given the discrepant findings reported for fat intake and incident BMLs, we suggest that this should be explored in different study samples, with and without clinical knee OA. Interventional trials are also an option as they will better control for unmeasured confounders. This could be done in both animal and human studies. To conclude, the current available evidence indicates that fat plays a role in BML development; however, the direction of the effect is unclear.

A recent study by Davies-Tuck et al (197) found that serum cholesterol and triglycerides were associated with an increased incidence of BMLs in asymptomatic women. In the current study we did not find any associations between total cholesterol, triglycerides, and BMLs; however, we did find that HDL cholesterol was protective against

BML change. In a separate analysis with incident BMLs only, we found HDL cholesterol was also protective against incident BMLs, providing consistent evidence that HDL cholesterol may have positive effects on BML pathology. It has been previously postulated that atheromatous vascular disease may contribute to the development of OA through its effects on subchondral bone (191). Subchondral bone ischemia may be one mechanism by which vascular pathology contributes to the development of BMLs (192). HDL cholesterol is considered to be protective against vascular pathology through cholesterol transport, anti-inflammatory and anti-oxidant effects (264) and therefore may help to reduce BML development and progression.

In this study we were able to examine the effect of both increasing and decreasing BMLs, which other studies in the past have not been able to do (190, 197). The results were independent of potential confounders such as smoking, cardiovascular diseases, diabetes, ROA and statin use. However, there are some limitations to this study. First, a 2-D assessment of BMLs was made, using the slice with the greatest BML size. This may bias towards shallow but flat lesions. The majority of previous studies also grade BMLs on the slice with the greatest BML size; however, they use a semi-quantitative scale (0–3) rather than an areal measure. We acknowledge that our measure of BMLs is only a surrogate measure of volume. Recent methods have been developed to measure BML volume using a autoregression model (250, 251). It is our view that the slice thickness (4 mm) and interslice gap (0.5–1.0 mm) of our imaging protocol was too large to estimate volume with sufficient accuracy. Although our areal measure may contain some measurement error, it has been demonstrated to be more sensitive to change over time compared with a semi-quantitative measure of BMLs (265). The sensitivity of ordinal and areal BML measurements was recently tested in a randomised controlled trial (RCT) examining the effectiveness of zoledronic acid (ZA) on knee pain and knee BMLs. We found that a single infusion of ZA was effective in reducing pain intensity and BML size compared to placebo after six months (265). This effect was only seen with the areal BMLs and not ordinal BMLs, showing that an areal BML measure is more sensitive to change over time. Second, baseline and follow-up MRIs were read paired with the chronological order known to the observer. This may result in a small tendency to read more change in comparison with a blinded reading. However, it has been shown that scoring blinded to the chronological sequence significantly decreases sensitivity in the detection of clinically relevant changes in comparison with scoring in chronological order (266, 267). These studies showed that blinding to time point leads to misclassification of change in a feature and that it may compromise the assessment of the relation of that feature and its outcome.

Third, we did not measure knee alignment, which has been shown to be associated with BMLs (151, 165). Lastly, due to a small number of incident BMLs (n = 14) we were unable to stratify by clinical knee OA in order to better compare to previous studies.

In conclusion, energy, carbohydrate and sugar intake may be risk factors for BML development and progression. HDL cholesterol appears to have protective effects on both incident and increasing BMLs. These findings suggest macronutrients and serum lipids may be important in BML etiology. There are multiple mechanisms by which diet and lipids could contribute to BML pathology and further investigation into the relationship between diet, lipids, and BMLs is warranted.

Chapter 9 - Summary and future directions

9.1 Summary

Osteoarthritis (OA) is the most common joint disorder in the world and in Western populations is one of the most frequent causes of pain, loss of function, and disability in adults (268). With the aging population and the rise in obesity rates, the social and economic burden associated with OA is increasing. Despite its large disease burden, there are no proven preventative strategies and no treatments which stop or delay the progression of the disease. Therefore, conventional treatment of OA is mostly palliative and costly. Until recently, cartilage degeneration was considered to be the initial pathologic defect in OA. However, it is now recognised that bone changes occur early in the disease process and there have been some suggestions that bone changes may precede cartilage damage. This thesis has examined prospective associations between subchondral bone and disease progression and disease severity, and has presented novel and important findings which are summarised below.

Chapter 4 examined the cross-sectional correlates of subchondral bone mineral density (sBMD). Many factors were found to be associated with sBMD including age, sex, body mass index (BMI), vitamin D, sun exposure, and physical activity. Numerous knee structural features measured on x-ray and magnetic resonance imaging (MRI) were also associated with sBMD, such as tibial osteophytes, joint space narrowing (JSN), cartilage defects, cartilage volume, and bone marrow lesions (BMLs). Of these, the one that is most interesting is cartilage defects which were found to be independently associated with sBMD. Although causation cannot be drawn from this, it appears that denser subchondral bone may increase the risk of defect development. sBMD was also positively associated with cartilage volume and we hypothesise that cartilage swelling is an early manifestation of OA. A longitudinal study was required to examine whether elevated sBMD is a risk factor for cartilage defect development and cartilage volume loss, which prompted the work done in Chapter 5. Lastly, there was a modest correlation between sBMD and BMD at the hip and spine, which suggests that sBMD may be under independent regulation to bone at other sites.

Chapter 5 describes the relationship between sBMD and bone area with cartilage damage. This prospective study supported the hypothesis generated from the previous cross-sectional analysis in Chapter 4. Elevated sBMD at baseline was associated with medial cartilage defect development. However, sBMD was not associated with lateral cartilage defect development or medial or lateral cartilage volume loss. This study also supported previous research demonstrating that bone area predicts both medial and lateral

cartilage defect development. To the best of our knowledge, this was the first study to demonstrate that bone area predicted cartilage volume loss. This association was seen at the medial site only. The associations presented between sBMD and bone area with cartilage damage were independent to each other, suggesting there are multiple mechanism by which subchondral bone changes may lead to changes in cartilage.

Chapter 6 examined the site-specific relationship between BMLs and cartilage damage. Baseline BMLs predicted site-specific cartilage defect progression and cartilage volume loss. This suggests that BMLs may be having a local effect on cartilage homeostasis. This is the first study to show that baseline cartilage defects predicted BML progression. This relationship was also site-specific, which suggests that increased bone loading, due to cartilage defects, may result in BMLs. There was a greater increase in cartilage volume loss when both larger cartilage defects and BMLs were present. Altogether these findings indicate that BMLs and cartilage defects may not necessarily drive one another; although it is possible, they may co-occur in the pathway towards increased disease. BMLs and cartilage defects appear interconnected and play key roles in cartilage volume loss; thus, both should be considered targets for intervention.

Chapter 7 described the natural history of BMLs using a quantitative measure and examined the relationship between BMLs with pain and knee replacement surgery. In this population-based sample of community-dwelling older adults, 43% of participants presented with a BML at baseline. Of these, similar proportions were both worsening (24%) and improving (25%). The incidence of new BMLs in subjects who were BML free at baseline was low (7%). A change in BML size was associated with a change in pain, only in those without established radiographic osteoarthritis (ROA). Importantly, this was the first study to demonstrate that a reduction in BML size was associated with an improvement in knee pain (also only in those without established ROA). This suggests that fluctuations in knee pain may be attributable to BMLs in those with early stage disease. Lastly, exploratory analysis revealed that baseline BMLs predicted knee replacement surgery over 5 years. Overall, these findings highlight that BMLs are an attractive target for therapeutic intervention.

Chapter 8 examined the relationship between dietary factors, serum lipids, and BMLs. An elevated energy, carbohydrate, and sugar intake at baseline were positively associated with a change in BML size. It is likely that increased energy is the major contributor to BMLs and carbohydrate and sugar intake could be surrogate measures for increased energy intake. A high energy diet is associated with increased free radical production and oxidative stress (257, 258) and this may be having an effect on the

subchondral bone. This study also reported that baseline high-density lipoprotein (HDL) cholesterol is protective against both incident and increasing BMLs. This is in line with the hypothesis that atheromatous vascular disease may contribute to the development of OA through its effects on subchondral bone (191). HDL cholesterol is protective against vascular pathology through cholesterol transport, anti-inflammatory and anti-oxidant effects (264) and therefore may help to reduce BML development and progression. Overall these findings support a systemic role in BML pathology.

In conclusion, this series of related analyses of a prospective population-based study of community-dwelling older adults provides considerable insight into the role subchondral bone plays in OA. Most importantly, these results highlight that subchondral bone is an attractive target for therapeutic intervention. Recommendations for the future direction are provided in the following section.

9.2 Future directions

This thesis has presented several novel findings from a large prospective study of older community-dwelling adults. The most important implication is that BMLs are an attractive target for intervention. Chapters 6 and 7 examined the role that BMLs play in disease progression and disease severity. BMLs predicted important disease outcomes such as cartilage defect development, cartilage volume loss, and joint replacement surgery and have the potential to regress and resolve. They are also linked to knee pain, an important clinical outcome. An increase in BML size was associated with an increase in knee pain. Similarly, a decrease in BML size was associated with an improvement in knee pain. This relationship was only present in those without established ROA, suggesting that fluctuating knee pain may be a result of BML changes in those with early stage disease.

Several studies have evaluated the effects of the antiresorptive agents, bisphosphonates, on OA. Using a guinea pig OA model, Meyer et al (269) found that the pyridinyl bisphosphonate risedronate was shown to slow disease progression, as measured by the size and severity of cartilage lesions and the size of osteophytes, by up to 40%. Spector et al (270) showed trends towards improvement in both radiographic joint structure and symptoms. They hypothesised that bisphosphonates reduce local bone turnover, decreased bone mineral content and stiffness, and decreased the trabecular number observed in OA subchondral bone structure. In a separate study, Bingham et al (271) found no treatment effect of risedronate on knee symptoms or radiographic progression; however,

there was little decline in the placebo group, thus no way to tell if risedronate had an effect or not.

Recently bisphosphonates have been shown to be a potential candidate to treat BMLs as there is observational evidence that BMLs are less common in persons taking alendronate (272). A study investigating the effect of risedronate on cartilage loss in knee OA suggested that risedronate 50 mg weekly may prevent an increase in BML size (167), although this was not statistically significant. Based on the results from this thesis, we have recently conducted a randomised controlled trial (RCT) examining the effectiveness of zoledronic acid (ZA) on knee pain and knee BMLs. This study was a single centre double blind placebo controlled randomised trial of intravenous (IV) ZA (5 mg) vs placebo in 59 adults aged 50–80 years with knee pain (>40 mm on a visual analog score (VAS)) and a knee BML. We found that a single infusion of IV ZA was effective in reducing pain intensity and BML size compared to placebo after six months (265). This study is an important step in identifying treatments which may slow the progression of knee OA, as we have demonstrated both improvement in pain and structure. Future work is required to examine the effect over longer timeframes and in larger samples, and to evaluate the effect of different doses.

Other potential agents for modifying disease progression in OA include glucosamine sulphate, chondroitin sulphate, sodium hyaluronan, doxycycline, matrix metalloproteinase (MMP) inhibitors, calcitonin, diacerein, inhibitors of inducible nitric oxide synthase, bone morphogenetic protein 7 (BMP-7), vitamin D, collagen hydolyzate, fibroblast growth factor, interleukin (IL)-1 inhibitors, and avocado-soybean unsaponifiables (ASUs). These compounds are at different stages of development and have varying routes of administration and different mechanisms of action.

It remains unclear whether decreasing BML size will translate to reductions in cartilage loss or rates of knee replacement over time. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is used to assess cartilage glycosaminoglycan (GAG) distribution. GAG concentrations in articular cartilage can change quickly; therefore, dGEMRIC would be a useful tool to determine if altering BML natural history improves cartilage biochemistry. Additionally, assessment of cartilage breakdown markers, such as type II collagen C-terminal telopeptide (CTX-II), is required. Other agents with known potent effects on osteoclasts could also be considered for future work.

Although BMLs are proving to be an attractive target in OA, more work is needed to develop a better understanding of BMLs and the best way to treat them. Chapter 7 examined the natural history of BMLs and found that in the Tasmanian Older Adult Cohort

(TASOAC) population, BMLs were not static with similar proportions both worsening (24%) and improving (25%). Previous studies examining BML natural history, which have mostly been performed in symptomatic OA cohorts, have shown conflicting results. For example, one study reported that <1% of patients showed a BML decrease over 30 months (165); while, in contrast, another study found that 20% of BMLs decreased over 2 years (177). The reasons behind these variations remain unclear but one possibly may be the histological heterogeneity of BMLs. BMLs consist of several abnormalities including bone marrow necrosis, abnormal trabeculae, bone marrow fibrosis, bone marrow bleeding, bone marrow edema and sclerosis (152, 157). BML resolution may occur in some histological profiles but not others. The challenge is to identify which of these types will be responsive to therapy. In TASOAC, BMLs were measured on T2-weighted fat saturation sequences. It is accepted that this is one of the sequencing types which depicts non-cystic lesions to their maximum extent; however, correct assessment of sclerotic lesions require non-fat saturated sequences (273). Future work could combine image modalities and use both fat saturated and non-fat saturated sequences to better quantify changes in granulation, fibrosis, and sclerosis.

Chapters 4 and 5 examined the role sBMD plays in OA. We found that sBMD predicted medial cartilage defect development, suggesting a role for elevated sBMD in the origin of cartilage defects. No association was seen between sBMD and cartilage volume loss suggesting that sBMD may not be an important measure to predict disease progression in OA. However, cartilage defects are a major contributor to cartilage volume loss (145, 146) and longer time frames may be required to see cartilage defects attributable to increased sBMD translate to cartilage loss. Whether or not there is a future in monitoring sBMD in OA is unknown and further work is needed to confirm the role sBMD may play in cartilage loss. Treatments aimed at decreasing BMD would not be ideal as they could increase the risk of fractures.

Chapter 5 demonstrated that tibial bone area predicted both cartilage defect development and cartilage volume loss. This provides support that subchondral bone expansion is likely to be a primary event in OA. It was thought that the increase in tibial bone area was due to osteophyte formation (88); however, we now know that bone expansion due to increased bone area can occur in the absence of osteophytes (142). Therefore, it is suggested that increases in bone area most likely reflects knee joint surface expansion, which could indicate remodelling of the subchondral trabeculae due to increased extracellular matrix deposition as a result of excessive load on the bone in adult life (105, 274). Interventions aimed at reducing bone size and preventing bone area

expansion may have a role in reducing cartilage damage; however, such interventions have not been identified to date.

Chapter 8 supports a systemic role in BML pathology. Increased energy, carbohydrate, and sugar intake were associated with BML development and progression. This suggests that there may be role for dietary modification to alter BML natural history. However, the relationship between dietary factors and BMLs may not be causal, and it may be that factors associated with diet are the underlying cause. There is also conflicting data regarding the relationship between fat intake and BMLs. Surprisingly, we found that fat intake was protective against incident BMLs; whereas, Wang et al (190) reported that increased saturated fat intake predicted incident BMLs. Given these discrepant findings further prospective studies examining the relationship between fat and BMLs are needed. Our finding that HDL cholesterol is protective against both incident and increasing BMLs is novel. Overall Chapter 8 suggests a role for diet and cholesterol modification to alter BML natural history. Intervention trials would be ideal as they will better control for unmeasured confounders and could be done in both animal and human studies.

MRI will continue to be an important research tool in OA. There has been a rapid improvement in imaging technology over the last 20 years and this has translated to improved understanding of the importance of individual knee structures and their relation to clinical outcomes and disease progression (91, 275). The development of multiple scoring systems has led to improved quantification of these pathologies (276). However, there remains a lack of standardisation regarding MRI interpretation and little consistency in its diagnostic application (97). A recent meta-analysis (90) has concluded that OA changes on MRI can be measured reliably using both semi-quantitative and quantitative measures. Also, MRI can accurately and feasibly measure change in cartilage morphology over 12 months for knee OA (90). Although MRI has not been formally accepted by regulatory authorities (78, 91), this review (90) provides strong support for the inclusion of MRI structural change in regulatory guidance statements. Recently, a panel of experts in the field have developed 11 propositions for a definition of OA on MRI (97). These definitions will be formally tested in relation to their diagnostic performance. This is an important step for OA research and will help to create uniformity and consistency when using MRI to diagnosis disease status and severity and monitor progression. This will also lead to better evaluation of MRI as a tool in detecting the effects of potential disease-modifying interventions.

In conclusion, this analysis of data from a prospective population-based cohort study indicates that subchondral bone plays a significant role in OA pathogenesis. This

thesis supports a role for bone changes in the early stages of disease. Features of the subchondral bone contribute to knee pain and predict important disease outcomes such as cartilage volume loss and joint replacement surgery. Future work should consider subchondral bone treatments when developing disease-modifying OA drugs (DMOADs).

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Appendices

Appendix 1 – A pilot study of the reproducibility and validity of measuring knee subchondral bone density in the tibia (with permission from Elsevier)

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A pilot study of the reproducibility and validity of measuring knee subchondral bone density in the tibia

D. Dore BBiotech.(Hons.)*, C. Ding M.D. and G. Jones M.D.
Menzies Research Institute, University of Tasmania, Hobart, Australia

Summary

Objective: To describe the reproducibility and validity of six different measurement techniques for knee subchondral bone mineral density (sBMD).

Methods: A consecutive sample of 50 male and female participants from a population-based longitudinal study had sBMD assessed using dual energy X-ray absorptiometry scans. Anthropometric, knee pain, cartilage and bone measures by magnetic resonance imaging and radiographic osteoarthritis (OA) were assessed. The six methods were defined as: (1) the midpoint of one intercondylar spine, across the tibial surface and descending 10 mm; from the midpoint of the two intercondylar spines (2) the top of the spine descending 20 mm, (3) 10–20 mm beneath the top of the spine; from the tibial surface descending, (4) 10 mm, (5) 15 mm, and (6) 20 mm.

Results: All six methods had excellent reproducibility (intra-class correlation coefficient 0.98–1.00). sBMD was higher in males (methods 2–4) and higher in those with medial tibial osteophytes (methods 1, 3 and 4). Medial tibial cartilage defects and overall cartilage defects correlated with sBMD (methods 3 and 4). Method 2, which includes the intercondylar spine, correlated with medial tibial bone size. Measuring sBMD using methods 3 and 4 produced the greatest number of associations with joint features of OA.

Conclusions: These preliminary results need confirmation in larger longitudinal samples but suggest that sBMD can be accurately measured and plays a role in knee OA. Methods 3 and 4 had the best concurrent validity; however, method 2 adds additional information on tibial bone size, suggesting that two measures are necessary in clinical studies.

Key words: Osteoarthritis, Knee, Subchondral bone, Dual energy X-ray absorptiometry.

Introduction

Osteoporosis can be diagnosed by measuring bone mineral density (BMD) of the hip or lumbar spine using dual energy X-ray absorptiometry (DXA)[1]. BMD at these sites is an important predictor of vertebral deformities and fractures[1].

BMD may also be important for osteoarthritis (OA). Longitudinal studies have shown that a high systemic BMD is an important predictor of knee OA and the higher the BMD is, the greater the risk of incidence and progression of this disease[2]. Additionally, it has been found that patients with advanced stages of OA have elevated BMD levels at their osteoarthritic sites, as well as in their femoral neck and lumbar spine[3]. It has been hypothesized for many years that subchondral bone plays a key role in the development and progression of OA[4]; however, there are limited human studies which confirm this. Recently, we have reported that subchondral bone size does appear to have a role in the initiation of cartilage defects and meniscal extrusion which in turn leads to cartilage loss[5]. It is unclear if this is primarily due to the enlarged bone size or the increased BMD or both.

It is feasible to assess subchondral BMD (sBMD) in the tibia; however, a number of issues remain outstanding. Definitions of subchondral bone in the tibia have varied amongst researchers, making it difficult to select one particular measure when assessing sBMD. Currently there is no consensus on an appropriate region of interest (ROI) used to measure sBMD with DXA. There is also limited research on the reproducibility and validity (construct, concurrent and content) of measuring subchondral bone density.

The aim of this pilot study, therefore, was to test the reproducibility and validity of sBMD measurements using six different techniques in a consecutive sample of subjects taking part in a population-based longitudinal study.

*Address correspondence and reprint requests to: Dawn Amanda Dore, BBiotech.(Hons.), Menzies Research Institute, University of Tasmania, Private Bag 23, 199 Macquarie Street, Hobart, Tasmania 7000, Australia. Tel: 61-362-267736; Fax: 61-362-267704; E-mail: dawn.dore@utas.edu.au

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Materials and methods

SUBJECTS

This study was conducted as part of the Tasmanian Older Adult Cohort study (TASOAC), an ongoing prospective, population-based study that began in 2002. Men and women between the ages of 50 and 79 years were randomly selected from the electoral roll in Southern Tasmania. Subjects who were institutionalized were excluded from the study. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants. The current study consists of a consecutive sample of 50 participants enhanced for radiographic knee OA.

ANTHROPOMETRICS

Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707). Height was measured to the nearest 0.1 cm (with shoes and

socks removed) using a stadiometer. Body mass index (BMI) was calculated as kg/m^2.

KNEE PAIN

Participants were asked to quantify their knee pain using the Western Ontario and McMaster Universities OA Index (WOMAC) pain score. Five categories of pain (walking on flat surface, going up/down stairs, pain at night, sitting/lying and standing upright) were assessed separately with a 10-point scale from 0 (no pain) to 9 (most severe pain). Each score was then summed to create a total pain score (range 0–45)[6]. Prevalent knee pain was defined as a total score of $\geq$1.

X-RAY

A standing anteroposterior semiflexed view of the right knee was performed in all subjects. Radiographs were then assessed utilizing the Altman atlas[7]. Each of the following was assessed on a scale of 0–3: medial joint space narrowing (JSN), lateral JSN, medial femoral osteophytes, medial tibial osteophytes, lateral femoral osteophytes, and lateral tibial osteophytes. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas and were blinded to the subject's status of knee pain. Intra-observer reliability assessed as intra-class correlation coefficients (ICCs) ranged from 0.65 to 0.85 as previously reported[8].

MAGNETIC RESONANCE IMAGING (MRI)

MRI of the right knee was acquired with a 1.5 T whole-body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit–receive extremity coil. Image sequences included the follwing: (1) a T1-weighted fat saturation three-dimensional (3-D) gradient recall acquisition in the steady state, flip angle 30°, repetition time 31 ms, echo time 6.71 ms, field of view 16 cm, 60 partitions, 512 $\times$ 512-pixel matrix, acquisition time 5 min 58 s, one acquisition; sagittal images were obtained at a partition thickness of 1.5 mm without between-slice gap; and (2) a T2-weighted fat saturation 3-D fast spin echo, flip angle 90°, repetition time 3067 ms, echo time 112 ms, field of view 16 cm/15 partitions, 228 $\times$ 256-pixel matrix; sagittal images were obtained at a partition thickness of 4 mm with a between-slices gap of 0.5–1.0 mm.

Knee cartilage volume was determined by means of image processing on an independent workstation using Osiris software[9]. The volumes of individual cartilage plates (medial tibia and femora and lateral tibia and femora) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then re-sampled by means of bilinear and cubic interpolation (area of 312 $\times$ 312 mm and 1.5 mm thickness, continuous sections) for the final 3-D rendering.

Cartilage defects were assessed on the T1-weighted MR images and scored with a modification of a previous classification system at medial tibial, medial femoral, lateral tibial, and lateral femoral as follows[10]: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface; grade 2 = irregularities on the surface or basal layer and loss of thickness <50%; grade 3 = deep ulceration with loss of thickness >50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. It was found that a cartilage surface in some images was still regular but cartilage adjacent to subchondral bone became irregular, so it was included in the classification system. A cartilage defect also had to be present in at least two consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. The highest score was used if >1 defect was present on the same site. One observer scored the MRI blinded to knee pain score. Intra-observer reliability assessed as ICCs ranged from 0.80 to 0.95 at the medial femoral and tibial, and lateral femoral and tibial sites as previously reported[11].

Subchondral bone marrow lesions were assessed on the T2-weighted MR images and defined as discrete areas of increased signal adjacent to the subcortical bone at the lateral, medial femur and/or tibia. Each bone marrow lesion was scored on the basis of lesion size, e.g., a lesion was scored as grade 1 if it was only present on one slice, grade 2 if present on two consecutive slices, or grade 3 if present on greater than three consecutive slices. The highest score was used if greater than one lesion was present on the same site. Prevalent bone marrow lesions were defined as a total score >1. One observer scored the bone marrow lesions blinded to knee pain score. ICCs were 0.89, 0.96, 0.94, and 1.00 for the bone marrow lesions scores at lateral tibia, lateral femur, medial tibia, and medial femur, respectively[11].

The area of medial and lateral tibial plateau bone was measured manually on the three reformatted images closest to the tibial cartilage from axial T1-weighted fat-saturated 3-D MRI. This method is highly reproducible[12,13]. An average of these three areas was used as an estimate of the tibial plateau bone area[14].

DUAL ENERGY X-RAY ABSORPTIOMETRY

In this study, BMD was measured using a Hologic Delphi densitometer (Waltham, MA, USA) to determine BMD at the knee using existing spine software.

Subchondral bone was defined as the entire tibia proximal to the head of the fibula, which is in line with recent reports. Six different ROIs or "methods", as referred to in this study, were chosen to measure subchondral bone density using DXA. The six different ROIs for each method were drawn manually around the bone and are described in Fig. 1(A–F). These ROIs were chosen due to their particular features. Method 1 used the midpoint of only one intercondylar spine (either medial or lateral) and, therefore, excluded the midpoint of the tibia. This was chosen to capture sBMD directly beneath the medial or lateral area of loading. Method 2 was the only method which took into account the intercondylar spine. Method 3 was the only method which excluded the tibial plateau. Methods 4–6 included the tibial plateau and measured different ROI heights, i.e., 10, 15, or 20 mm. In addition, all of these methods follow protocols which have been used in related studies measuring sBMD with DXA.

DATA ANALYSIS

Using each of the six methods, medial and lateral sBMD measurements were repeated three times and an average of the three was taken to get a mean sBMD for each method. Reproducibility was calculated using ICCs. To test intra-observer reproducibility, ICCs were used to compare each of the three measurements, hence the name 'immediate reproducibility'. ICCs were also calculated between measures 1 and 2, 2 and 3 and 1 and 3 to determine whether or not multiple measurements were necessary for a more reproducible sBMD measurement.

Twenty of the same scans were re-read after a time interval of approximately 14 days and new ROIs were drawn based on the initial criteria. Due to time constraints and other results, only methods 2 and 3 were re-evaluated. Method 2 was considered to be important because it took into account the intercondylar spine. Method 3 was tested because it was considered to be a standard method of measurement. Interval intra-observer reproducibility was calculated as the ICCs between the immediate measurements and the re-read ones.

Medial sBMD was compared only with medial structural outcomes such as medial JSN, medial osteophytes, medial bone marrow lesions, medial cartilage defects, and medial bone size. Lateral sBMD was also only compared with lateral structural outcomes.

An unpaired t test was used to compare sBMD between dichotomous variables and the Pearson correlation coefficient, r, to compare continuous variables. The Spearman correlation coefficient, ρ, was used to calculate associations between sBMD and other non-continuous variables. Partial correlation analysis was used to examine associations after adjusting for age, sex, and BMI. A P-value < 0.05 (two tailed) was considered statistically significant. All statistical analyses were performed on SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

SUBJECTS

A total of 50 subjects (27 men and 23 women) with a mean age of 65 years were included in this study. The characteristics of the study population are presented in Table I. Prevalent knee pain (34%), JSN (52%), and osteophytes (58%) were common amongst participants. Tibial osteophytes were present in 17 subjects and not present in 33. Femoral osteophytes were present in 12 subjects and not present in 38. Twelve subjects had tibial and femoral osteophytes; five subjects had tibial osteophytes but no femoral osteophytes; and 33 subjects had no tibial or femoral osteophytes.

REPRODUCIBILITY

All six methods were found to have excellent immediate reproducibility, with ICCs ranging from 0.98 to 1.00 (Table II). The ICCs between measurements 1 and 2, 2 and 3 and 1 and 3 varied little and ranged from 0.97 to 0.99, for all six methods. The interval intra-observer reproducibility for methods 2 and 3 was not as good as immediate reproducibility with the exception of method 2 at the medial site which had an ICC of 0.96 (Table II).

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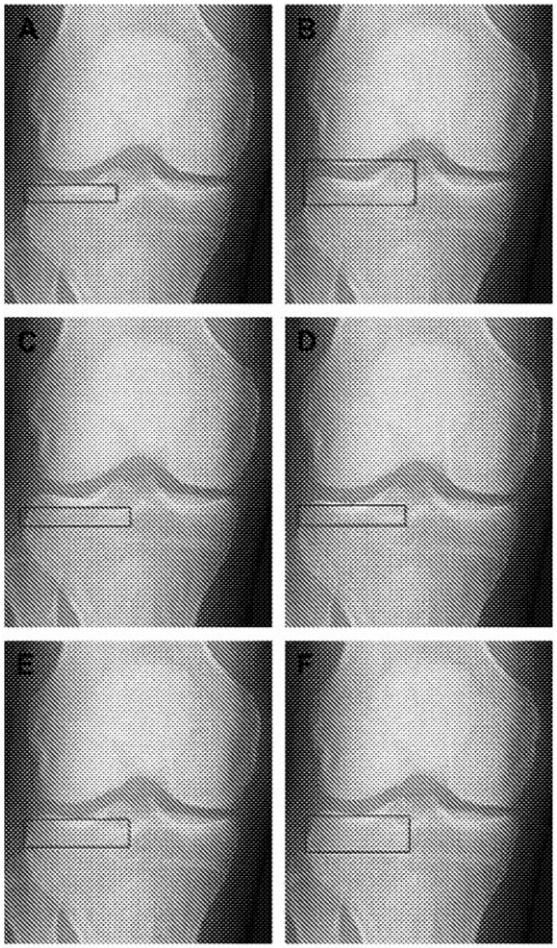

Fig. 1. Six ROIs. (A) Method 1 used the midpoint of either the medial or lateral intercondylar spine as a reference, the top of the ROI was set at the tibial surface and descended 10 mm, the width of the box extended to the edge of the image. Methods 2–6 use the midpoint of the two intercondylar spines as a reference. (B) Method 2; the top of the ROI was the highest point of the medial or lateral spine and extended to the edge of the image; either medially or laterally. The ROI was a height of 20 mm and the width of the tibial bone. This method included either the medial or lateral intercondylar spine. (C) Method 3; descended 10 mm down from the highest point of the medial or lateral spine. The ROI extended to the edge of the image; either medially or laterally. The ROI was a height of 10 mm (10–20 mm beneath the top of the tibial spine) and the width of the tibial bone. (D) Method 4; the top of the ROI was at the tibial surface and descended 10 mm; the width of the ROI extended to the edge of the image either medially or laterally. (E) Method 5; the top of the ROI was at the tibial surface and descended 15 mm; the width of the ROI extended to the edge of the image either medially or laterally. (F) Method 6; the top of the ROI was at the tibial surface and descended 20 mm; the width of the ROI extended to the edge of the image either medially or laterally.

DEMOGRAPHIC FEATURES

With methods 2–4 medial sBMD was higher in males. The mean differences for methods 2–4 were 0.07 g/cm^2 ($P = 0.010$), 0.10 g/cm^2 ($P < 0.001$), and 0.10 g/cm^2 ($P < 0.001$). Methods 1, 5, and 6 showed no significant sex difference in medial or lateral sBMD.

Table III summarizes the remainder of associations between demographic features and sBMD. There was no association between sBMD and age, weight, or BMI. There was a positive correlation between height and medial sBMD using methods 2–5.

Table I
Subject characteristics (N = 50)

Age, years	64.5 ± 7.1
Gender, % male	54
Height (cm)	166.4 ± 8.6
Weight (kg)	80.8 ± 17.1
BMI (kg/m^2)	29.1 ± 5.7
Any JSN, %	52
Any osteophytes, %	58
Any bone marrow lesion, %	25
Cartilage volume, mL	
Medial	2337 ± 785
Lateral	2587 ± 774
Tibial bone area, mm^2	
Medial	2012 ± 370
Lateral	1086 ± 231
Total defects, grade (median)*	
Medial	2.0
Lateral	2.0
Prevalent knee pain, %	34

Unless otherwise indicated, values are mean ± SD.
*Cartilage defects graded (0–4).

MEDIAL MEASUREMENTS

In adjusted analysis, sBMD using method 1 was only associated with tibial osteophytes ($r = 0.39$, $P = 0.006$). Associations between features of OA and sBMD measurements using methods 2–4 are summarized in Table IV.

Table II
Reproducibility of each method

	ICCs, measures of agreement	
	Initial measurement	Second blind measurement
Method 1		
Medial	1.00	–
Lateral	0.98	–
Method 2		
Medial	0.99	0.96
Lateral	0.98	0.49*
Method 3		
Medial	1.00	0.68
Lateral	0.99	0.79
Method 4		
Medial	1.00	–
Lateral	1.00	–
Method 5		
Medial	0.99	–
Lateral	0.99	–
Method 6		
Medial	0.98	–
Lateral	0.99	–

*$P = 0.08$, all other $P < 0.01$, second blind measurements were performed approximately 14 days after the initial measurements.

Table III
Association between demographic factors and subchondral bone density

Factors	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Age						
Medial	0.02	−0.13	−0.13	−0.01	−0.09	−0.18
Lateral	0.07	−0.03	0.02	0.05	−0.02	0.01
Height						
Medial	0.28	**0.55****	**0.35***	**0.40***	**0.32***	0.01
Lateral	0.24	0.28	0.23	0.20	0.06	0.08
Weight						
Medial	0.17	0.24	0.23	0.25	0.16	0.08
Lateral	0.04	0.13	0.07	0.05	−0.02	−0.11
BMI						
Medial	0.02	−0.01	0.07	0.05	−0.01	0.07
Lateral	−0.10	−0.02	−0.05	−0.05	−0.01	−0.16

Correlation coefficient, bold denotes statistically significant result, *$P < 0.05$, **$P < 0.01$.

Method 2 was the only method which had a significant correlation between sBMD and tibial bone size in a univariate analysis. The association between tibial bone size and sBMD remained significant after adjustment for age and BMI ($r = 0.49$, $P = 0.002$); however, this relationship did not persist after further adjustment for sex (Table IV). There was also no relationship seen when sBMD and bone size were stratified by sex [males $r = 0.16$ ($P = 0.518$) and females $r = 0.09$ ($P = 0.71$)].

sBMD was higher in those with tibial and femoral osteophytes using methods 1, 3 and 4 (Fig. 2). In adjusted analysis, sBMD was higher in those with tibial osteophytes using methods 3 and 4 (Table IV). sBMD was also higher in those with greater tibial cartilage defect scores and overall cartilage defect scores using methods 3 and 4 (Table IV). sBMD using method 3 correlated significantly with femoral cartilage defects (Table IV). Femoral osteophytes were positively associated with sBMD using method 4 (Table IV). Additionally, JSN correlated with sBMD using method 4 (Table IV).

In adjusted analysis there were no significant associations between sBMD and joint features of OA using method 5 or 6.

LATERAL MEASUREMENTS

There were fewer associations between lateral sBMD and lateral features of OA. In adjusted analysis there was a negative association between WOMAC pain and sBMD using method 2 ($r = -0.308$, $P = 0.035$) but this most likely reflects a chance association. sBMD from method 4 correlated with tibial osteophytes ($r = 0.425$, $P = 0.006$), femoral osteophytes ($r = 0.403$, $P = 0.009$), tibial bone marrow lesions ($r = 0.481$, $P = 0.001$), and femoral defects ($r = 0.313$, $P = 0.046$).

Discussion

This pilot study demonstrates that sBMD measurements of the tibia are reproducible and demonstrates validity. Method 2 plus either method 3 or 4 would appear best for further studies.

All six measures were found to be highly reproducible on immediate repeat measurement. No major advantage was evident with three measures over two suggesting two measures are sufficient. In general, with the exception of method 2 at the medial site, the ICCs for interval measurements were worse and will need improvement. This suggests that measures should be done at the same time or that methods should be developed to minimize drift in measurement such as an ROI atlas. Joint position may also affect these results but our data cannot test this issue because we did not attempt to measure re-positioning error. Given the interval ICCs, re-positioned scans are also likely to have less than desirable ICCs. However, this is also true for the MRI literature on cartilage and bone measurements and most authors accept this is a valid measure. In a comparable study on sBMD, Bruyere *et al.* tested the precision of seven ROIs[15]. They performed five measurements of sBMD from each of the seven ROIs. The patients had five separate DXA scans and the results showed a precision for the assessment of tibial sBMD, from 2.1 to 3.1% depending on the chosen ROI.

Methods 3 and 4 had the best concurrent validity having significant associations between medial sBMD and sex, height, tibial osteophytes, tibial defects and overall defects. Method 3 produced additional information on femoral bone marrow lesions and femoral defects; whereas, method 4 gave information on JSN and femoral osteophytes. Furthermore, both methods 3 and 4 showed that subjects with

Table IV
Associations between medial sBMD and joint pathology

Factors	Method 2		Method 3		Method 4	
	Uni-variable	Multi-variable*	Uni-variable	Multi-variable*	Uni-variable	Multi-variable*
JSN (0–3)	0.14	0.11	0.24	0.27	**0.35***	**0.41****
Tibial osteophytes (0–3)	0.19	0.16	**0.35***	**0.41****	**0.37****	**0.34***
Femoral osteophytes (0–3)	0.03	−0.08	0.22	0.26	0.28	**0.30***
Tibial bone marrow lesions (0–3)	0.24	0.15	0.20	0.26	0.09	0.10
Femoral bone marrow lesions (0–3)	0.03	0.01	0.19	**0.39***	0.07	0.15
Tibial defects (1–4)	0.28	0.15	**0.32***	**0.41****	**0.41****	**0.46****
Femoral defects (1–4)	0.18	0.08	**0.31***	**0.36***	0.30	0.28
Overall defects (1–8)	0.26	0.13	**0.38***	**0.41****	**0.40****	**0.40***
Cartilage volume	0.29†	−0.02	0.07†	−0.20	0.14†	−0.07
Tibial bone size	**0.48**,†**	0.10	0.30†	0.02	0.31†	−0.03
WOMAC pain	0.03†	0.02	0.01†	−0.03	−0.05†	−0.07

Values from medial assessment, bold denotes statistically significant result, *$P < 0.05$, **$P < 0.01$.
*Adjusting for age, sex, and BMI (Spearman correlation coefficient).
†Pearson correlation coefficient.

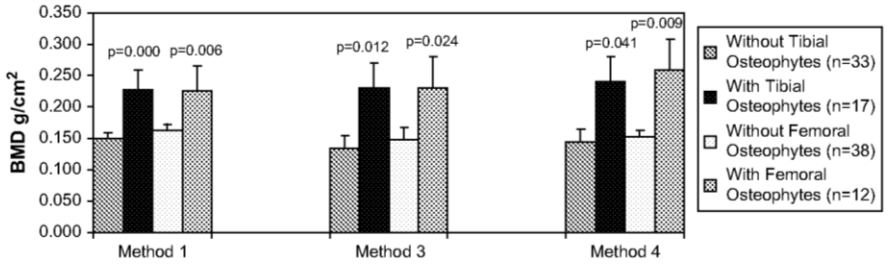

Fig. 2. Mean difference in medial sBMD between those subjects with and without medial tibial or femoral osteophytes using methods 1, 3, and 4. Error bars represent standard deviation (SD).

osteophytes had a significantly higher sBMD than those without osteophytes.

Using methods 1, 5 and 6 there was no association between sex and sBMD; however, there was an association using methods 2–4. This is most likely due to the small sample size of the study because sex differences in sBMD were large in magnitude varying between 12 and 92% for all the six methods of measurement.

Methods 4–6 were similar in positioning and only varied in height. Method 4 was 10 mm in height and methods 5 and 6 were 15 mm and 20 mm, respectively. Out of these three methods, method 4 had much greater concurrent validity, correlating with many joint features of OA. This suggests that when the height of the ROI starts to exceed 10 mm, there is a decrease in concurrent validity. This could be attributed to less relevance of subchondral bone for joint health below the 10 mm mark. It has been suggested that sBMD increases may be attributed to increased osteophytosis at the endplates of bone[2]. When deeper bone is included in the measurement, it could possibly alter the overall BMD in the subchondral region.

Somewhat surprisingly, method 2 was the only method which produced a significant correlation between medial sBMD and medial tibial bone size. Seeing as this method included the intercondylar spine, as well as the tibial plateau, it was expected that the association would be strongest for this measure. In a restricted analysis adjusting for age and BMI, it was found that tibial bone size correlated with sBMD using method 2. However, in further analysis adjusting for sex, the relationship did not persist. This could be due to the fact that adjusting for sex may alter the results because males have bigger bone area and a higher BMD. There was also no relationship seen when sBMD and tibial bone size were stratified by sex; however, the numbers are small in each subgroup so this result is potentially misleading. The tibial bone size association is largely mediated by larger body size in males so that similar results are observed if height and weight are used instead of sex (data not shown).

Tibial cartilage defects (methods 3 and 4), femoral cartilage defects (method 3), and overall cartilage defects (methods 3 and 4) were associated with a high sBMD. Ding *et al.* demonstrated that tibial subchondral bone area expansion predicts cartilage defects which then in turn predict cartilage volume loss[5]. It is unclear if this is primarily due to the size of the bone or the density or both.

There were fewer associations between lateral sBMD and lateral features of OA. Knee OA more commonly involves the medial tibiofemoral compartment and changes are often more pronounced in the medial tibial femoral joint rather than on the lateral side[16]. In our study subjects displayed a greater prevalence of medial joint pathology than lateral which is the most likely explanation for the difference between associations for medial vs lateral measurements. However, these differences may also be real and may contribute to site differences in knee OA. Larger studies will be needed to resolve this issue and the current study will inform the design of such studies.

There are a number of potential limitations to the current study. First, the height of the ROI was not adjusted to account for the absolute proportions of individual knees and will have captured different proportions of bone depending on bone size. However, the width of each ROI went to the edge of the bone (either medially or laterally); therefore, varied according to individual knee sizes. Second, the cross-sectional nature of this study precludes any inference about cause and effect relationships. Longitudinal studies will be required to sort out these issues. Third, sBMD provides an assessment of the bone at the knee, it only evaluates bone in two-dimensions and thus only approximates volumetric density. Other techniques will be required to assess this. Fourth, we did not correct for chance in the data analysis; however, it is our standard practice (which is based on the epidemiology literature) not to adjust for multiple comparisons but to present all comparisons in the paper so that the reader can assess this. In this case, there have been many more significant associations than would be expected by chance. Lastly, the small sample size of this pilot study may have affected the ability to detect modest associations between sBMD and age and weight. The negative association should not be regarded as definitive. Overall, this study should be regarded as hypothesis generating as it was an exploratory study.

In conclusion, these preliminary results need confirmation in larger longitudinal samples but suggest that sBMD can be accurately measured and plays a role in knee OA. Methods 3 and 4 had the best concurrent validity; however, method 2 adds additional information on tibial bone size, suggesting that two measures are necessary in clinical studies.

Conflict of interest

Nothing to declare.

Acknowledgments

A special thanks goes to the subjects who made this study possible. The role of Sr Catrina Boon in collecting the data is gratefully acknowledged. We would like to thank

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Appendix 2 – Example of the pedometer diary used in TASOAC

TASOAC PEDOMETER DIARY Phase 2 Round 1

Name.....TASOAC ID☐☐☐☐☐☐ Pedometer number☐☐☐

Sensitivity number.....☐ **Remember to reset Pedometer Daily.**

Day	Date	Start time	End time	Display number	Time spent NOT wearing pedometer Whilst participating in physical activity	Name activity participated in whilst not wearing pedometer	List any circumstances which may have affected pedometer reading
1							
2							
3							
4							
5							
6							
7							

Appendix 3 – Example of the nutrient intake report from the food frequency questionnaire (FFQ)

Scan ID	Scan Date	Portion Standard Factor	Energy kJ/day	All Fat g/day	SatFat g/day	PolyFat g/day	MonoFat g/day	Protein g/day	Carbohydra g/day	Sugars g/day	Starch g/day	Fibre g/day	Alcohol g/day
1948	7/03/2005	0.725	6699.821	61.1701	30.63372	7.717802	17.76102	63.03661	203.0659	101.7313	100.3643	19.41932	0

BetaCar ug/day	Calcium mg/day	Cholesterol mg/day	Folate ug/day	Iron mg/day	Magnesium mg/day	Niacin mg/day	Niacin Equi mg/day	Phosphorus mg/day	Potassium mg/day	Retinol ug/day	Retinol Equ ug/day	Riboflavin mg/day	Sodium mg/day
2148.3	1061.274	214.5715	243.2412	10.54445	262.4491	14.83017	28.15072	1273.983	2433.896	451.9157	809.9382	2.077933	1948.391

Thiamin mg/day	VitaminC mg/day	VitaminE mg/day	Zinc mg/day	Alpha-Carc ug/day	Beta-Carot ug/day	Beta-Crypt ug/day	Lutein+Zea ug/day	Lycopene ug/day	FD40 g/day	FD60 g/day	FD80 g/day	FD100 g/day	FD120 g/day
1.3519	84.1606	4.772141	8.098256	724.1287	2825.672	163.2716	710.828	1333.864	0.8125666	0.442995	0.521081	0.8767341	2.298161

FD140 g/day	FD150 g/day	FD160 g/day	FD170 g/day	FD180 g/day	FD200 g/day	FD220 g/day	FD240 g/day	FD141 g/day	FD151 g/day	FD161 g/day	FD161T g/day	FD171 g/day	FD181 g/day
4.3079	0.3415976	16.05117	0.305939	9.46227	0.286424	0.121433	7.23E-02	0.3307192	4.09E-03	0.891477	0	3.89E-02	21.2029

FD181T g/day	FD201 g/day	FD221 g/day	FD182N6 g/day	FD182N6T g/day	FD183N3 g/day	FD202N6 g/day	FD203N6 g/day	FD204N6 g/day	FD205N3 g/day	FD205N6 g/day	FD224N6 g/day	FD225N3 g/day	FD226N3 g/day	Glycemic Load	Glycemic Index
0.679	0.1650253	4.26E-02	6.578168	0.0749	0.949695	1.96E-02	1.07E-02	2.56E-02	2.39E-02	0	2.85E-03	9.64E-03	0.061914	110.3033	54.31897

Appendix 4 –Subsequent correspondence regarding Chapter 5: Letter to the editor by Hayashi et al and our reply (this material is reproduced with permission of John Wiley & Sons, Inc.)

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LETTERS

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Choice of pulse sequences for magnetic resonance imaging–based semiquantitative assessment of cartilage defects in osteoarthritis research: comment on the article by Doré et al

To the Editor:

We would like to comment on magnetic resonance imaging (MRI) assessment of focal cartilage defects in osteoarthritis (OA) research from a radiologic point of view. Doré et al recently reported the association between baseline tibial bone area and tibial subchondral bone mineral density (BMD) with tibial cartilage defect development and cartilage volume loss (1). They concluded that bone area predicted medial and lateral cartilage defect development and medial cartilage volume loss, while subchondral BMD predicted medial defect development, but not cartilage loss. Tibial cartilage defects were graded on a scale of 0–4 using T1-weighted fat suppressed 3-dimensional (3-D) gradient-recalled acquisition in the steady-state sequence, which is one of several gradient-recalled echo (GRE) type of sequences. GRE-type sequences such as 3-D spoiled gradient-recalled acquisition in the steady-state (SPGR), fast low-angle shot, 3-point Dixon, and double-echo steady-state are well suited for depicting the cartilage volume, and thus they are the sequence of choice for quantitative (volumetric) analysis of cartilage (2,3).

However, for depicting subtle cartilage abnormalities, GRE-type sequences are less suited than fluid-sensitive sequences (such as fat-suppressed intermediate [IM]–weighted, proton density [PD]–weighted, or fast spin-echo [FSE] T2-weighted image sequences [4,5]). With fat-suppressed IM-, PD-, and T2-weighted FSE sequences, normal hyaline cartilage has intermediate signal intensity, and intraarticular fluid is bright and displays good contrast due to an "arthrographic" effect that identifies surface abnormalities as well as abnormalities in cartilage matrix (6) (Figures 1A and B). Moreover, GRE-type sequences are very susceptible to artifacts (motion artifacts from long imaging time and susceptibility artifacts), leading to difficulty differentiating between true cartilage defect and signal changes due to artifact (Figures 1C and D).

Recent studies applying MRI semiquantitative assessment of focal cartilage defects use fluid-sensitive sequences (7–9) rather than GRE-type sequences because of these difficulties. Doré and colleagues' choice of MR sequence for assessment of focal cartilage defects is therefore not appropriate, according to the latest reports that advocate using these techniques. The authors should have at least acknowledged these facts in their report.

As a consequence of the inadequate choice of pulse sequences in their study, it is unclear what the results presented by this group pertaining to cartilage defects represent. Detection of lesions and the estimations of their size will be inaccurate, so adequately interpreting the results and drawing conclusions based upon them seems impossible. We would strongly encourage others to use appropriate MRI pulse sequences to evaluate focal cartilage abnormalities, and if there is uncertainty, to seek advice from adequately trained musculoskeletal radiologists when developing studies, in order to achieve highest possible image quality with currently available technologies.

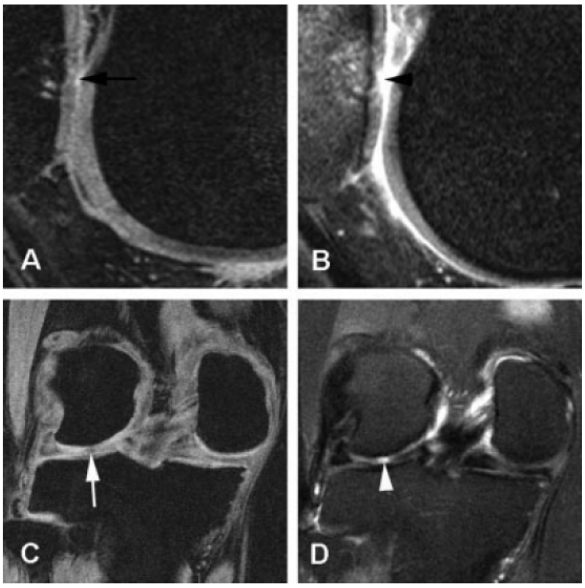

Figure 1. **A** and **B**, Focal superficial defect in the retropatellar articular surface. Sagittal double-echo steady-state (T2-weighted gradient-recalled echo [GRE]–type sequence) image barely depicts surface abnormality (**A**) (**arrow**). Due to arthrographic effect, the defect is better visualized on sagittal intermediate-weighted fat-suppressed image (**B**) (**arrowhead**). **C** and **D**, Full thickness defect in the central weight-bearing region of the lateral femur. Coronal fast low-angle shot (T1-weighted 3-dimensional GRE-type sequence) image shows discrete hypointensity in the cartilage matrix, not to be assessed as a defect, that could potentially be read as an artifact such as truncation or susceptibility (**arrow**) and slight motion artifacts can be noted, due to long imaging time (9 minutes 35 seconds) (**C**). Coronal T2-weighted fat-suppressed image clearly depicts full-thickness defect, due to excellent contrast between cartilage and intraarticular fluid (**D**). (Images in **A** and **B** from the Osteoarthritis Initiative study and **C** and **D** from the Framingham Osteoarthritis study.)

Dr. Roemer owns stock in BICL. Dr. Guermazi is the president of Boston Imaging Core Lab (BICL). He owns stock in Synarc, Inc. and has received consulting fees from MerckSerono, Facet Solutions, Genzyme, and Stryker (less than $10,000 each).

Daichi Hayashi, MBBS
Boston University
Frank W. Roemer, MD
Ali Guermazi, MD
Boston University
and Boston Imaging Core Lab
Boston, MA

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Reply

To the Editor:

We appreciate the opportunity to reply to Hayashi and colleagues. They have commented on MRI assessment of focal cartilage defects in OA from a radiologic perspective. Although the quality of images is important, we would like to respond from an epidemiologic point of view.

Hayashi and colleagues make the forceful claim that we utilized an inappropriate choice of sequences in assessing cartilage defects with MRI. Specifically, they argue that GRE-type sequences are less suited than fluid-sensitive sequences such as fat suppression IM-weighted, PD-weighted, or T2-weighted FSE sequences to assess cartilage defects. This is largely based on comparative studies performed in animal models or in human cadaver ankle models. Although some of these studies have suggested that FSE-type sequences are qualitatively superior, most have shown very similar overall diagnostic performance, with no statistically significant differences between the 2 sequence types. One study performed in participants with knee OA showed that images from both FSE- and GRE-type sequences showed similar agreement (kappa 0.601 and 0.583, respectively) with arthroscopy (1). Furthermore, GRE-type sequences have been shown to be accurate and reliable for the detection of cartilage defects, with sensitivity and specificity ranging higher than 90% in some studies, as compared with arthroscopic results (2–4). Therefore, we disagree with the statement by Hayashi and colleagues that our choice of MR sequence for assessment of cartilage defects is not appropriate according to the available evidence.

We acknowledge that our assessment of cartilage defects may entail some measurement error and misclassification, but this is true for virtually all measures in epidemiology. For example, physical activity assessed by questionnaire has a weak correlation with actual physical activity, but is still widely used in epidemiologic studies due to its convenience and lower respondent burden. However, any measurement error and misclassification using our measure of cartilage defects is likely to be random and would dilute the effects that are seen, thus reducing the ability to detect significant findings (5). It would not result in spurious associations. As is important for any measure, the reproducibility and reliability of our method is high in multiple settings (6–9), and there is a moderate correlation with histologic findings (10). Most importantly, our measure of cartilage defects has also been shown to be associated with clinically important outcomes such as pain, radiographic OA, cartilage volume loss, cartilage breakdown markers, bone marrow lesions, and knee replacement surgery (11) in multiple cross-sectional and longitudinal studies. Cartilage defects in these studies have been assessed by different trained readers who were blinded with regard to clinical data, demonstrating high methodologic quality; for a cartilage defect to be identified it also had to be present on 2 consecutive slices, further reducing measurement error. We have also shown that our method of assessment is sensitive to change over time (8). Additionally, it is convenient and cost-effective to use the same sequences for assessing both cartilage defects and cartilage volume. None of these benefits have been shown for the image sequence proposed by Hayashi and colleagues, and, in the absence of such evidence, their assertion of methodologic superiority is insufficiently supported. Comparative studies of predictive validity for clinical outcomes with results supporting their view are needed before such strong statements should be made.

The statement that interpretation of the results from our recent report seems impossible is surprising to us. An artifact would have none of the measurement characteristics we described above if detected by our method; rather, it would be random and unrelated to any important outcomes, whereas the consistency of our results using this measure in different settings is striking. Furthermore, in our study, we found that bone area predicted both cartilage defect increases and cartilage volume loss, demonstrating face validity. Hayashi and colleagues reference a review by Link (12) which states that GRE-type sequences are more susceptible to artifacts; however, it is made clear by the author that this is a consideration in patients who have had previous surgery, and in particular cartilage repair procedures, which does not apply to our population-based studies.

To conclude, we thank Hayashi and colleagues for the opportunity to discuss the important epidemiologic issues regarding measurement in clinical and epidemiologic studies, which include not only imaging, but also validity, reproducibility, clinical relevance, and the potential impact of any measurement error. We maintain that the results of our recent

study of cartilage defects, measured using GRE-type sequences, are valid. Furthermore, this discussion has highlighted that in both clinical medicine and epidemiology studies there is often more than one way by which to measure a structure or feature, so it is critical that investigators and readers consider the epidemiologic issues around measurement integrity when interpreting study findings. We politely suggest that, based on the current evidence, there should not be any specific recommendations in regard to the best MRI protocol for obtaining images from which cartilage defects can be scored in population-based studies.

D. Doré, BBiotech
T. Winzenberg, MD
University of Tasmania
Hobart, Tasmania, Australia
C. Ding, MD
University of Tasmania
Hobart, Tasmania, Australia
and Monash University
Melbourne, Victoria, Australia
F. Cicuttini, PhD
Monash University
Melbourne, Victoria, Australia
G. Jones, MD
University of Tasmania
Hobart, Tasmania, Australia

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Selective estrogen receptor agonism lacks clinical benefit in rheumatoid arthritis: comment on the article by van Vollenhoven et al

To the Editor:

I read with interest the article by van Vollenhoven et al on selective estrogen receptor agonism and would like to respond by reporting our group's findings in the studies of the role of estrogens in rheumatoid arthritis (RA) in relation to the estrogen receptor (ER) agonism treatment (1).

Based on findings in animal models, van Vollenhoven and colleagues undertook a study to determine the effects of the selective ERα agonist Org 37663 in terms of both its estrogenic effects and its ability to ameliorate disease activity. They studied 164 patients with RA and administered Org 37663 at a dosage of 4 mg/day, 15 mg/day, or 50 mg/week, along with background therapy of methotrexate (MTX) or sulfasalazine for 10 weeks. In several organ systems, Org 37663 induced clear biologic, estrogenic responses, including a dose-related increase in levels of sex hormone binding globulin. However, the Disease Activity Score in 28 joints (2) decreased similarly in all treatment groups including placebo, indicating a lack of clinical efficacy of Org 37663 in this trial. Therefore, the authors concluded that the presence of a biologically relevant ERα-mediated estrogenic effect was not associated with a clinically relevant benefit on the symptoms and signs of RA. The authors partially explained the absence of the expected clinical effects by suggesting that ERα may not be the predominant estrogen receptor in humans; ERβ may actually be of greater importance, and they reported that recent data have indicated that ERβ is involved in regulating inflammation in rats and mice (1).

Recently, our group investigated the efficacy and safety of an ERβ agonist (ERB-041) in 291 RA patients with active disease, who were treated with stable doses of MTX and randomized to receive 5 mg/day, 25 mg/day, or 75 mg/day of ERB-041, or placebo for 12 weeks (3). The primary end point was the ACR 20% improvement criteria (ACR20) (4) at 12 weeks. Secondary measures assessed included ACR50 and ACR70 responses, health outcomes measures, C-reactive protein (CRP) levels, and potential exposure-response relationships. Similar to the findings in the study with ERα agonist, no statistically significant difference in the ACR20 was found between ERB-041 treatment and placebo groups ($P = 0.518$). Neither was a significant difference observed for ACR50 and

Appendix 5 – Example of the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index

Rate the following today for KNEES

3.2. This section assesses pain, stiffness and functional deficit on a scale from 1 - 10

Example	none									severe
Example of no pain	● 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
Example of severe pain	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	● 10

1. Referring to your knees only how much pain do you experience when

	none									severe
a. Walking on a flat surface	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
b. Going up and down stairs	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
c. At night while in bed	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
d. Sitting or lying	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
e. Standing upright	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

2. Referring to your knees only how much stiffness do you experience

	none									severe
a. After first awakening	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
b. Later in the day	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

3. Referring to your knees only how much functional deficit do you experience when

	none									severe
a. Descending stairs	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
b. Ascending stairs	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
c. Rising from bed	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
d. Rising from sitting	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
e. Putting on socks	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
f. Taking off socks	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
g. Bending to the floor	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
h. Lying in bed	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

Question 3 continued	none									severe
i. Walking on flat surface	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
j. Getting in/out of the bath	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
k. Standing	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
l. Sitting	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
m. Getting in/out of the car	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
n. Getting on/off the toilet	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
o. Heavy domestic chores	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
p. Light domestic chores	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
q. Shopping	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10